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Calcium physiology and terrestrial ecosystem processes

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SUMMARY

Calcium occupies a unique position among plant nutrients both chemically and functionally. Its chemical properties allow it to exist in a wide range of binding states and to serve in both structural and messenger roles. Despite its importance in many plant processes, Ca mobility is low, making Ca uptake and distribution rate a limiting process for many key plant functions. Ca plays an essential role in regulating many physiological processes that influence both growth and responses to environmental stresses. Included among these are: water and solute movement, influenced through effects on membrane structure and stomatal function; cell division and cell wall synthesis; direct or signaling roles in systems involved in plant defense and repair of damage from biotic and abiotic stress; rates of respiratory metabolism and translocation; and structural chemistry and function of woody support tissues. Forest trees, because of their size and age capacity, have been examined for evidence of limitations imposed by the timing and level of Ca supply. Examination of Ca physiology and biogeochemical cycling for forested systems reveals many indications that Ca supply places important limitations on forest structure and

function. These limitations are likely to be most significant with older trees, later successional stages, high levels of soil acidity and/or high canopy Ca leaching losses, or under conditions where plant competition is high or transpiration is limited by high humidity or low soil moisture. Evidence of structural and physiological adaptations of forests to limited Ca supply; indicators of system dysfunction at many levels under reduced Ca supply; and the positive responses of diverse indicators of forest vitality in liming experiments indicate that Ca is more important to forest function and structure than has generally been recognized. Lack of recognition of Ca limitations is due in part to that fact some important plant functions are controlled by changes in very small physiologically active pools within the cytoplasm, and whole-leaf Ca levels may not reflect these limitations. An additional aspect is the fact that Ca availability has declined significantly for many forests in just the past few decades. Additional research on the role of Ca supply in resistance of forests to disease, changes in structural integrity of woody tissues, restrictions on rooting patterns and function, and uptake of other nutrients, notably N, is needed. Increased understanding of the physiological ecology of Ca supply can be anticipated to provide important insights that will aid in future protection and management of both natural and commercial forest systems.

Key words: calcium, forests, ecosystem, physiology, structure, function.

1. INTRODUCTION – A HYPOTHESIS

The diverse functions of Ca in regulating physiological and structural processes in both animals and plants are now widely recognized after decades of research. Ca is unique among the elements comprising living systems because of its role as a messenger for many different types of physiological processes and its unique chemical binding properties (Hepler & Wayne, 1985). For these reasons Ca plays an important role in adding stability and structural integrity to biological tissues at scales ranging from intercellular membranes to the cell walls of woody stems. Reviews over the past 15 years have documented steady progress in the definition of an increasingly broad array of responses and mechanisms by which Ca functions to influence plant processes (Bangerth, 1979; Clarkson & Hanson, 1980; Ferguson, 1984; Kirkby & Pilbeam, 1984; Rorison & Robinson, 1984; Hepler & Wayne, 1985; Kauss, 1987; Pooviah, 1988; Roberts & Harmon, 1992; Bush, 1995; McAinsh *et al.*, 1996; Webb *et al.*, 1996; Trewavas & Malho, 1997; McAinsh & Hetherington, 1998).

The number of processes in which Ca plays an important regulatory role is impressive, including phosphorylation of nuclear proteins; cell division; cell wall and membrane synthesis and function; intra- and intercellular signaling; protein synthesis; responses to environmental stimuli, including low temperatures, gravity, insects and disease; stomatal regulation; and carbohydrate metabolism. The diversity of these processes is even more remarkable with respect to higher plant function when it is recognized that, among the primary plant macronutrients, Ca is the least mobile, making the issue of Ca supply and conservation critical to growth and development in resource-limited environments.

The need to place Ca metabolism in the broader perspective of factors that determine its availability in the external environment (Bangerth, 1979) and affect its distribution within the plant (Kirkby & Pilbeam, 1984) has become increasingly important in recent years. There are two reasons for this: first, a

wealth of recent research on Ca metabolism and signaling in plants has made additional understanding of the nature and potential connectivity of Ca-regulated processes at the whole-plant level increasingly possible; and second, because there are increasing indications that the activities of humans in recent decades have reduced the availability of Ca in terrestrial ecosystems.

Environmental influences that produce significant spatial and temporal gradients in Ca supply are of interest from the perspectives of both plant species and plant community development. These influences include changes in regional scale atmospheric deposition of strong acids, which have long been recognized as a factor that increases Ca loss from forests (Overrein, 1972); large-scale reductions in atmospheric deposition of Ca to terrestrial ecosystems (Hedin *et al.*, 1994); and whole-tree harvesting, which can remove important stores of Ca in foliage and branches from both temperate (Johnson *et al.*, 1988; Federer *et al.*, 1989) and tropical forests (Jordan & Herrera, 1981). Biogeochemical responses of interest include changes in patterns of Ca uptake, retention, and cycling at organizational scales ranging from cell membranes (DeHayes *et al.*, 1997) to forested watersheds (Johnson & Lindberg, 1992; Federer *et al.*, 1989; Ulrich & Matzner, 1986).

Studies of nutrient cycling in terrestrial ecosystems at scales ranging from multispecies laboratory microcosms (Van Voris *et al.*, 1980) to watersheds (Federer *et al.*, 1989) indicate that loss of Ca is an important early indicator of disturbance of nutrient cycling in terrestrial ecosystems. Recent documentation of significant long-term losses of Ca in soils and vegetation within the Hubbard Brook Watershed in the northeastern USA (Likens *et al.*, 1996, 1998) indicates that issues of Ca supply and cycling might provide increasingly important insights into future patterns of forest growth and ecosystem function.

20 years ago Bangerth (1979) emphasized that many factors in the environment ultimately affect Ca concentration in plant organs, and he called for

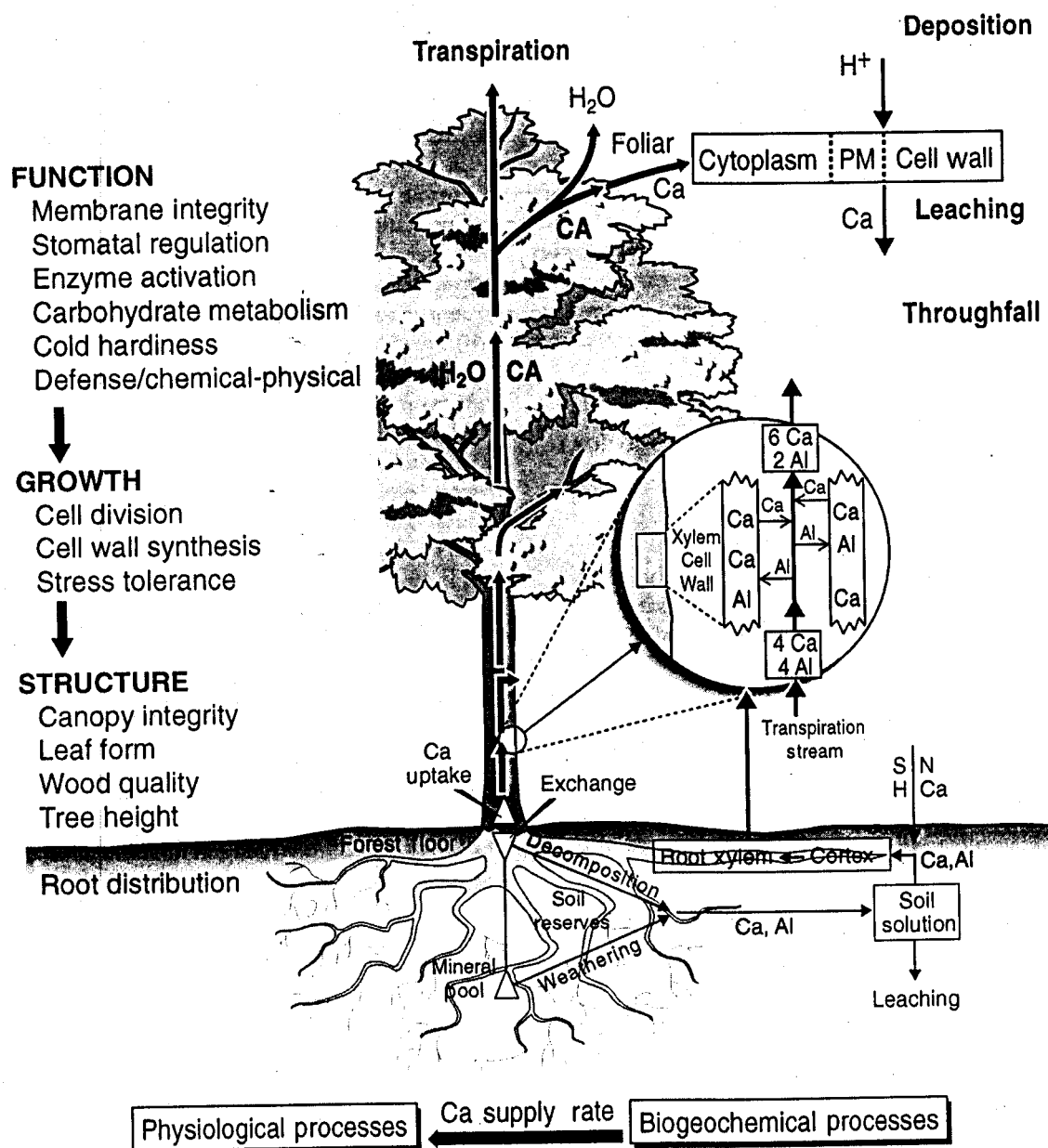


Fig. 1. Diagram showing indicators of forest physiological function, growth and structure that are linked to biogeochemical cycles through processes that control rates of Ca supply.

extending the field of experimentation in Ca metabolism into more complex environmental settings. He wanted field experiments '... to define which factors are decisive for Ca uptake and distribution.' He further noted that 'Such experiments are far more laborious and expensive than investigations in controlled environments, but they are necessary for a more efficient utilization of the already accumulated information on the basic mechanisms of Ca uptake, distribution, and function.' Many types of such experiments have now been performed, sometimes by design, and sometimes by chance, as research has increasingly focused on understanding and predicting plant and ecosystem responses to disturbance and to the impacts of current and projected future changes in chemical and physical climate. These

experiments have provided a format for examining a wide array of processes potentially influenced by Ca supply levels along both spatial and temporal gradients.

Here, we propose a hypothesis that stems from two previously noted aspects of the metabolic functions of Ca: regulation of many critical metabolic processes; and limited mobility in plants. Our hypothesis is that Ca supply exerts a significant control on both the structure and the function of forest ecosystems. We reason that for a nutrient element having these properties, adaptive strategies to limitations in Ca supply will have had an important influence on the physiology, morphology, growth and evolutionary development of individual plant species as well as the differentiation of plant

communities. We propose that mechanisms for Ca conservation will be increasingly evident on both physiological and structural adaptations in forests at the later successional stages as availability becomes increasingly limited. Our primary emphasis is on forest systems because their longevity, diversity and structural complexity make them excellent testing grounds for evaluating this hypothesis. Within forest systems we will focus on the interplay of Ca supply and Ca flux between compartments as they influence physiology, growth and nutrient cycling at organizational scales ranging from the cell to forest structure and function (Fig. 1).

II. EFFECTS OF CALCIUM ON PHYSIOLOGICAL PROCESSES

1. *The chemical uniqueness of calcium*

Fundamental to an appreciation for the varied roles that Ca plays in plant systems is an understanding of the unique chemical properties that allow it to exist in a wide variety of binding states, and thereby to fulfill diverse biochemical functions. Hepler & Wayne (1985) have provided an excellent overview of the chemical attributes that define the 'fitness of Ca' to fill these diverse roles. Here, Ca chemistry is considered in relation to three principal attributes: cytotoxicity; binding properties; and stimulation/displacement potential.

(a) *Cytotoxicity*. Ironically, this highly functional regulator of cellular processes is also highly toxic to cytoplasmic processes at the millimolar concentrations that exist outside the cytoplasm. This toxicity is a consequence of the precipitation of inorganic phosphate, which impairs phosphorous metabolism within the cell. Maintenance of the micromolar concentrations necessary for cell functions in the cytoplasm is accomplished by energy-requiring Ca transporters, which function within the plasma membrane, as well as within other membrane systems that delimit other subcellular organelles (Kauss, 1987). These pumps maintain a balance between Ca export from the cytoplasm by active transport through antiports and influx through specific channels (Bush, 1995). As a result, cytoplasmic concentrations are maintained in the micromolar range (10^{-8} – 10^{-5} M), but Ca levels in the other subcellular organelles, notably the vacuole, are in the millimolar range, a gradient of three to four orders of magnitude.

The end result of the steep concentration gradients that exist within the cell is that the potential for Ca gradients to be promoted by external stimuli is very high, and that the magnitude of change required in membrane systems of the subcellular organelles to affect significantly cytoplasmic Ca levels is very low (Bush, 1995). The active transport of Ca within the cell, resulting in the maintenance of steep

intracellular gradients, is in striking contrast to the more passive processes by which Ca moves from the soil into the plant through the xylem and the general lack of internal retranslocation at the organ level. For this reason, plant growth is very sensitive to changes in Ca supply rates, and estimates of Ca sufficiency for small seedlings under steady-state supply in nutrient culture (Goransson & Eldhuset, 1995) may underestimate the amounts required to maintain optimal growth under variable supply and demand sequences typically experienced in the field.

(b) *Binding properties*. The diversity of binding conditions that Ca can occupy is a consequence of its large nuclear size, charge density, and the availability of outer-shell electrons. These electrons allow Ca to participate in multiple configurations, including both covalent and ionic bonding. Because of its size and low charge density, Ca is specifically favored in bonds with neutral oxygen sites in the biological ligands, such as polysaccharides and lipids, and forms relatively weak bonds at multiple bonding sites. In comparison to its close competitor, the smaller, more charge dense, Mg ion, Ca has a relatively low affinity for water and N atoms, and is bound more readily to oxygen at the multiple binding points in cell membranes and cell walls. A low affinity for binding to water provides two advantages for Ca in biological systems: an enhanced capacity to bind to less highly charged ligands, because removal of water reduces the energy available for bonding; and fast reaction times, because Ca can quickly 'shed' water and become actively involved in alternate binding states. The release of water by Ca ions is approximately 100 times faster than for Mg, for example. The capacity to occupy multiple binding sites also allows Ca to be bound in much more complex configurations than Mg, perhaps explaining its capacity to be bound to proteins, and as a site-specific enzyme cofactor (Roberts & Harmon, 1992).

(c) *Stimulation/displacement potential*. Bush (1995) refers to 12 types of stimuli that cause changes in cytosolic Ca concentrations in various plant systems. These include a wide diversity of stimuli, such as low temperature, touch, gravity and the plant growth regulators auxin, cytokinin, ABA and GA. Most of these stimuli are accompanied by changes in cytosolic pH, indicating that exchange of hydrogen for Ca in cell membranes is involved in the signaling process (Bush, 1995). As an example, ion displacement of Ca in root membranes has been shown to occur in the order $Al > H > Fe > Mn$ (Stienen & Bauch, 1988); thus, the fluxes of these acidic cations through cell walls of fine roots influence the dynamics of retention and release of Ca, and the functional integrity of associated cell membranes (Zhao *et al.*, 1987). This is an important concept in addressing changes in Ca availability in developing plant communities, because normal patterns in forest

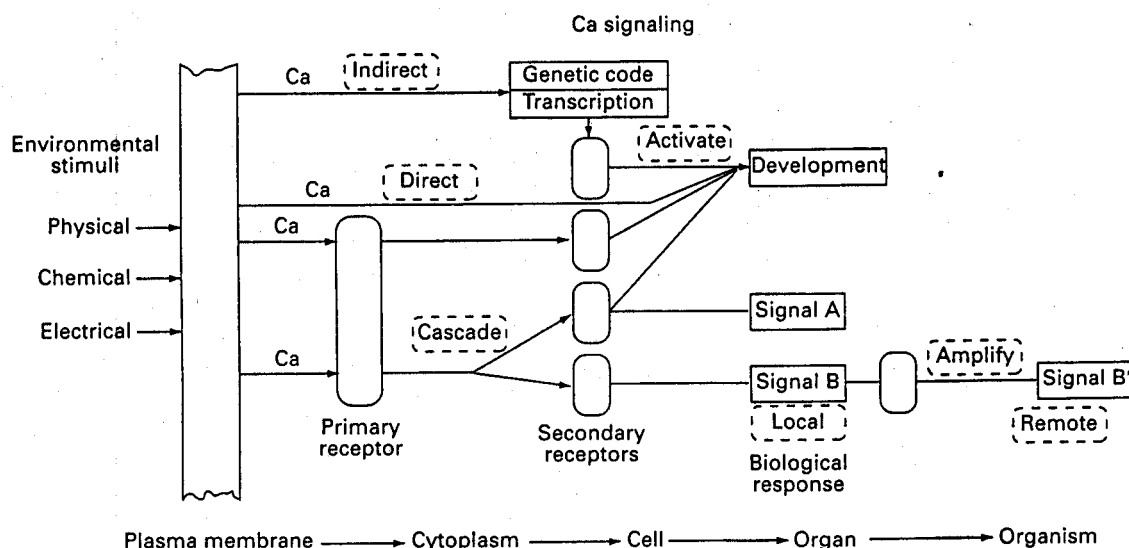


Fig. 2. Conceptual diagram of the types and sequences of signals involved in the Ca-mediated activation of receptors, which control diverse physiological functions in plants. Both direct and indirect control, involving primary and secondary receptors operating in both local (intracellular) and remote control functions are envisioned (see also Trewavas & Malho, 1997).

succession, as well as many human influences on those patterns, lead to diminishing availability of the basic cations, such as Ca and Mg, and increasing availability of acidic cations, such as H, Al, Fe and Mn in the soil environment at later successional stages (Ulrich & Matzner, 1986).

2. Calcium signaling and plant responses to environmental stress

(a) *Control principles.* Three keys to Ca regulation of plant processes are the maintenance of a homeostatic background of regulated compartments of Ca between the cytosol, the plasmalemma and the intracellular organelles; sensitivity of those compartments to external chemical and physical stimuli that create Ca impulses within the system; and a stimulus-response system by which Ca signals are translated into the plant response. The first two equip the plant system to respond to environmental stimuli; the last provides the signal by which a response occurs.

Significant advances in understanding of the diversity, timing and functional complexity of Ca signaling in plants have been made in the past decade as new Ca-specific dyes have allowed temporal and spatial sequences at the intracellular level to be detailed (Webb *et al.*, 1996). This has led to important new conceptual advances related to how Ca-induced signals are generated and transmitted in plants and how such responses are translated into the physiological responses that govern form and function (Trewavas & Malho, 1997; McAinsh & Hetherington, 1998; Snedden & Fromm, 1998). Here, we focus on conceptual highlights that relate to the timing and specificity of signals and their relationship to whole-plant physiology.

In Fig. 2, we have detailed the principal functional interrelationships among types and sequences of signals and responses involved in Ca signaling at the cellular level (Trewavas & Malho, 1997). Critical features of those responses can be summarized as follows:

- **Plasma membrane.** The plasma membrane plays a central role in transmitting Ca signals and in maintaining homeostasis. A series of ports and gates help regulate Ca flow and maintains the three order-of-magnitude gradients between cytoplasmic and membrane Ca contents. Other organelles have their own signaling systems such that Ca serves as an integrator of many types of information flow among organelles.
- **Signals and noise.** Ca signals can be produced by a variety of external stimuli and recognized as specific stimuli by internal receptors or combinations of receptors that elicit responses. The recognition that some types of signals occur as oscillations, with both a specific frequency and amplitude, provides an easy analogy to encoding in radio waves, which can be AM (amplitude-modulated) or FM (frequency-modulated), or perhaps both, in plant systems. Such signals can also have filters to remove noise and establish thresholds, as well as secondary amplifiers and transmitters to select and enhance relevant portions of the induced signals. A simple model of the translation of Ca oscillations to signal enzyme activation in plants is shown in Fig. 3. A central feature of this scheme is Ca regulation of protein phosphorylation, and alteration of the balance between phosphorylation and dephosphorylation of proteins that controls enzyme-regulated reaction kinetics.
- **Receptors.** Ca-activated proteins represent an important class of receptors for Ca-moderated

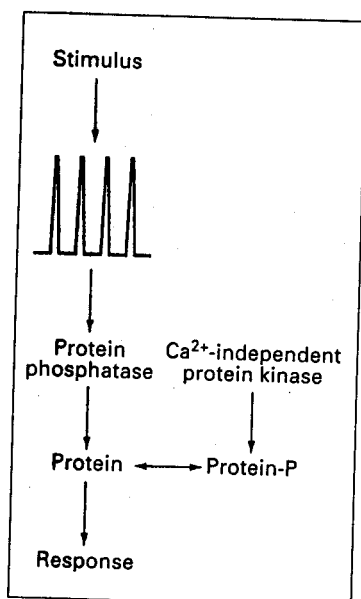


Fig. 3. A model for decoding the information contained in Ca spikes and oscillations that comprise signals in cytosolic free Ca levels. Such signals are envisioned to control relative rates of phosphorylation and dephosphorylation and hence activation levels of enzymes (McAinsh & Hetherington, 1998).

responses in plants (McAinsh & Hetherington, 1998). The identification of Ca-modulated proteins and their modes of action has proceeded rapidly in recent years, and Roberts & Harmon (1992) noted that over 150 such proteins have been identified. The most well characterized group of messenger proteins for Ca-induced signals are members of a Ca-binding family known as calmodulin-binding proteins (Snedden & Fromm, 1998). Calmodulin is ideally suited to serve as a Ca-activated signal because of its high solubility, low molecular weight, and the fact that it changes in charge (and conformation) upon binding with Ca (Kauss, 1987). Although calmodulin has no activity on its own, the role of messenger is exerted through its capacity to activate other enzymes. Thus, Ca-activated calmodulin can serve as part of an information cascade, directing activities of secondary systems at locations remote from the primary site of action. The identification of multi-gene families of calmodulin and calmodulin-related proteins, and the growing list of known targets of calmodulin, suggest that a complex Ca-based regulatory network controls a wide variety of responses to the environment (Snedden & Fromm, 1998).

● **ROM and RAM.** Trewavas & Malho (1997) coined a useful computer-based analogy to relate programmable translation systems for the variable Ca-specific signals produced by external environmental stimuli ('random-access memory', RAM) to the 'read-only memory' (ROM) by which plants are genetically shaped and biochemically constrained. Thus, the functionally more-complex responses that lead to plant growth and development can be

envisioned to involve increasing levels of coordination between genetic 'ROM' and environment-stimulated 'RAM'.

● **Ca movement and storage as signals.** The importance of Ca concentration to cell signals and cell growth has apparently led to built-in controls that help dampen the influences of '... the vagaries of the xylem source' (Trewavas & Malho, 1997). These include reservoirs in the membranes themselves, as well as accumulations as Ca-oxalate in specialized cells, the ideoblasts, from which subsequent solubilization can occur in response to localized Ca depletion. Thus, the size and distribution of these pools can provide some 'memory' of previous exposure, as well as providing buffering against subsequent changes in Ca supply. Additionally, it is now recognized that Ca signals can be transmitted as chemical waves within the plant. The geometry of conducting pathways and anatomical structures can create variable hydraulic pressure fields that influence these waves. Because the primary movement of Ca in plants is along longitudinal axes of water flow, such waves provide some interesting possibilities for integrating the influence of flow and structure to help coordinate phyllotaxis in plants.

In the following sections we provide examples of the importance and diversity of Ca signals operating in various direct and indirect ways to influence selected processes that are important to whole-plant function and associated responses to stress.

(b) *Carbohydrate metabolism.* Through its activation of a wide variety of protein kinases (Fig. 3), Ca has the capacity to influence many aspects of carbohydrate metabolism. However, the effects of Ca on carbohydrate metabolism have been more frequently related to its influence on respiratory metabolism and growth than to energy capture in photosynthesis. Indirect Ca-related control of respiration has been attributed to the loss of cellular compartmentalization and reduced membrane integrity associated with Ca deficiency (Bangerth, 1979), although the specific mechanisms of this control are not well characterized.

At supraoptimal levels of Ca supply, plants have a well-developed Ca immobilization system involving the formation of Ca-oxalate, which precipitates, and hence detoxifies, excess Ca. The formation of Ca-oxalate deposits can be shown to occur in both foliage (Fink, 1991) and woody stems of trees, particularly those growing in Ca-rich environments (Gourlay, 1995). Fink (1991) found insoluble Ca-oxalate crystals in Norway spruce (*Picea abies*) needles outside the mesophyll cell walls in which soluble Ca was bound as Ca pectate in the middle lamella. The diversion of photosynthetically fixed carbon to produce oxalic acid represents an energy sink that will divert some fraction of carbon and energy to this protective role. Oxalic acid is a major constituent of the TCA respiratory cycle in plants

and occurs in crystalline deposits at levels comprising up to 50% of some plant tissues (Ranson, 1965). Thus, it should be available in amounts adequate to immobilize excess Ca when the need arises.

The more widely documented example of the function of Ca in carbon metabolism is its capacity to repress respiratory activity of fruits. Respiratory activity is typically enhanced in fruits that suffer from poor Ca supply (Bangerth, 1979). Suppression of respiratory metabolism and delayed ripening of fruits by Ca have led to the use of Ca sprays to retard senescence and diseases of fruits in horticulture (Ferguson, 1984). Though less widely studied, Ca deficiency can also increase the respiratory rates of leafy tissue of herbaceous crops (Pal *et al.*, 1973) and forest trees (McLaughlin *et al.*, 1991, 1993). Rather modest reductions in foliar Ca have also been shown to reduce translocation of photosynthate from foliage of soybean plants (Gossett *et al.*, 1977).

The deterioration of a broad spectrum of essential physiological processes in Ca-deficient plants has led to recognition of the role of Ca supply in delaying plant senescence (Pooviah, 1988). Symptoms of Ca deficiency that are commonly associated with senescence include loss of protein, loss of chlorophyll, and reduced integrity of cell membrane and cell wall systems. Thus, the maintenance of adequate supplies of Ca will be particularly important with perennial species such as forest trees, where partitioning of carbon between growth, respiration and defense functions becomes increasingly important with age and the associated increasing respiratory demands on whole-tree energy budgets (McLaughlin & Shriner, 1980; Lechowicz, 1987; Waring, 1987).

(c) *Synthesis and function of membranes and cell walls.* Ca is essential for the structural integrity of membranes and cell walls because of both its activation of enzymes involved in structural biosynthesis (Brummel & MacLachlan, 1989) and its stabilizing influence on structural and functional attributes of these structures (Jones & Lunt, 1967). Membrane architecture and function is dependent on structural and spatial interrelationships between lipid and protein membrane components that regulate transmembrane flow of both nonpolar and polar materials (Palta & Li, 1978). Ca helps stabilize membrane structure through the formation of Ca-dependent hydrated crosslinks within the more polar regions (Minorsky, 1985). Leaching or displacement of Ca by exposure to increased atmospheric or soil acidity has been shown to lead to altered permeability of root membranes (Zhao *et al.*, 1987), while foliar leaching by acid mists can affect membranes responsible for regulation of water and electrolytes in foliage of conifers (Eamus *et al.*, 1989). Alteration of membrane structure by Ca deficiency leads to accumulation of Ca in vesicles, which is a typical symptom appearing in senescent cells (Hecht-Bucholz, 1979; Pooviah, 1988).

Ca influences the composition of cell walls in higher plants through its effect on the activities of the synthesizing enzymes. Activation is effected by movement of terminal complexes on the surface of the plasma membrane to form microfibrils. New fibril layers are sequentially deposited between the plasmalemma and the already-formed microfibrils. Modifications of cell wall composition are related to the relative activities of enzymes involved in the cell wall formation processes, including the glycosyltransferases, which are activated by Ca (Brummel & MacLachlan, 1989). Differentiation between cellulose and callose formation in the cell wall-forming process is also controlled by Ca, with greater relative amounts of callose, a wound-repair agent, being preferentially formed at the higher Ca levels (Delmer & Amor, 1995).

Wood formation in forest trees is influenced by Ca supply rates, both as a function of changes in cell wall plasticity of initiating cambial cells, as well as through stabilization of newly initiated cell walls during the growth process (Demarty *et al.*, 1984). Cambial cell walls change structurally and chemically as they go through alternating active and resting stages during the year. The shift of cambial cells from active to resting is accompanied by wall thickening and stiffening processes (Catesson, 1990). By contrast, the resumption of activity in the cambium of ash (*Fraxinus excelsior*) was accompanied by localized lysis of the radial walls (Catesson, 1990; Funada & Catesson, 1991). Because cross-linking of acidic pectins by Ca bridges is an important factor of wall rigidification, the breakdown of Ca-linked pectin molecules in the cambial zone appears to be a necessary prerequisite to increasing cell wall plasticity and the resumption of radial expansion of cambial cells (Funada & Catesson, 1991).

The initiation of cell division at the cambium begins with fusion of Golgi vesicles to form a cell plate consisting mainly of pectin. The principal component of pectin is a partially methylated α -1,4-polygalacturonic acid, and some of its carboxyl groups form Ca salts to produce a hard gel by cross-linking between the molecules. On both sides of this pectin layer, primary and secondary walls are formed successively. Therefore, pectic substances constitute one of the major carbohydrates of the intercellular portion in developing wood cells and are a major component of the primary wall of dicots (O'Neil *et al.*, 1990). Pectin is later degraded or removed prior to the lignification, apparently releasing Ca ions that were utilized during lignification (Westermarck *et al.*, 1986).

Dünisch *et al.* (1998) found K to be the driving force in cell enlargement of differentiating spruce tracheids (primary wall phase), while Ca is especially important for the secondary wall synthesis and lignification. This process of lignification starts in

the cell corner regions and proceeds in the middle lamella region after the primary wall has formed, and just before the formation of the secondary wall has started. Pectin has an important role during lignification in the cambial region and Ca plays a major role in this process. Westermarck (1982) suggested that lignin in wood cells is polymerized within the cell wall by a Ca-dependent mechanism. Ca-mediated peroxidase activity is involved in lignin synthesis, gradually converting the hydrophilic gel of the primary wall into a hydrophobic gel, comprised of lignin and hemicellulose, and releasing Ca.

The strength and flexibility of cell walls is determined by the biochemical composition, wall thickness, and stabilizing cross linkages. Mechanical flexibility of wheat coleoptile tissue was found to be reduced at high Ca supply resulting in a more rigid cell wall with reduced plasticity (Tagawa & Bonner, 1957). The assumption is that high Ca concentrations link and stiffen the polysaccharides of the wall, thereby preventing the turgor-driven extension from occurring. Cleland & Rayle (1977) argue that the inhibition of growth by high-tissue Ca content results more from an inhibition of biochemical wall-loosening processes than a direct stiffening action of Ca through formation of Ca-bridges.

(d) *Disease resistance and wound repair.* The association of Ca nutrition with plant resistance to a wide variety of diseases and physiological disorders is a well-documented result of the important structural and biochemical roles that Ca plays in defense and repair (Bangerth, 1979; Kirkby & Pilbeam, 1984; Pooviah, 1988). In addition, Ca functions as an early-warning signal aiding in the recognition of pathogen invasion (Dixon *et al.*, 1994). In evaluating the role of Ca in plant defense, it is important to consider both how Ca deficiencies develop spatially and temporally within the plant, as well as where and how Ca is specifically involved in the defense process.

Shear (1975) has listed over 30 Ca-related disorders that affect fruit and vegetable crops, including both deficiency and toxicity responses. Most were associated with foliage and fruits and represent a wide variety of rots and decays, and abnormal patterns of tissue growth, that become more abundant at low Ca concentrations. Diseases of fruits and vegetables have been of obvious interest from an economic perspective, but are also significant physiologically because they often represent the problems associated with poor Ca distribution rather than overall low Ca levels in the plant (Kirkby & Pilbeam, 1984). The apparent cause of the high sensitivity of fruits and vegetables to Ca-related diseases is that they are non-transpiring organs, without direct linkages to supply by transpiration (Clarkson & Hanson, 1980).

Increasing Ca supply to fruit trees with liming or direct application with various Ca-containing sprays is now well established as a means of remediating

diseases of fruits in the horticultural industry (Zocchi & Mignani, 1995; Raese & Drake, 1996). Stark (1964) cites numerous studies demonstrating that Ca fertilization can increase tree resistance to forest insects or adversely affect insect survival. Although Bangerth (1979) noted that Ca deficiencies can sometimes occur on plants growing in Ca-rich soils, and that liming is not always the answer, it appears evident from many studies that increasing Ca supply from the soil generally improves Ca distribution and the alleviation of many types of diseases. Two important components of plant defense, recognition and containment, which are dependent on Ca signals, are also influenced by Ca supply.

Rapid recognition and response to stress from wounding or disease can be an important measure of the likelihood that a plant will survive that damage. Davies (1987a) lists three types of wound signals produced at the site of injury to plant tissues. These include induction of chemical signals, such as hormones, oligosaccharides, or pheromones; electrical signals, which result in an electrochemical gradient or action potential that can alter polarity of plant membranes; and physical signals, such as plugging of plasmodesmata, changes in cytoplasmic streaming and changes in cell turgor that shift the balance of apoplast-symplast relationships.

Plant responses to these wound signals include both chemical and physical defenses that isolate the wound area and/or retard development of the pathogen (Dixon *et al.*, 1994). Rapid death of cells at the point of injury or attack – the hypersensitive response – can provide fast, effective protection by isolating the damaged area. Ca has been shown to be required for the hypersensitive response in soybeans and to be released in response to a wound-induced burst in H_2O_2 (Levine *et al.*, 1996). Plant release of antimicrobial agents, such as phytoalexins and hydrolytic enzymes, contribute by attacking or retarding the pathogen chemically. In addition, the formation of protective physical barriers with lignin, cell wall proteins, or callose (Dixon *et al.*, 1994; Davies, 1987a) may essentially wall-off the damaged or infected tissue. The formation of protective barriers is particularly important as a defense mechanism with forest trees (Shigo, 1984).

Although it is clear that plant defenses against injury and disease are multicomponent systems, which are both influenced by many aspects of host and pathogen physiology and modified by the environment, it is also clear that Ca plays a major role in several important aspects of this process. This includes serving as a primary intracellular signal in response to ion leakage produced by injury (Dixon *et al.*, 1994), as well as acting as a secondary intracellular signal induced by intercellular action potentials (Davies, 1987b). In addition, Ca has been shown to be required for synthesis of phytoalexin, an antimicrobial agent (Dixon *et al.*, 1994). Its signaling

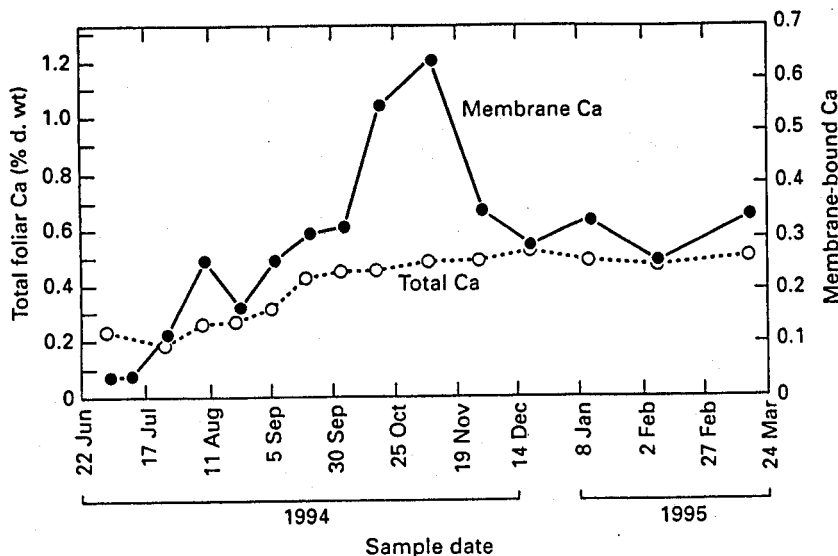


Fig. 4. Seasonal changes in membrane-associated Ca in current-year red spruce foliage show significant shifts associated with phenological changes and winter hardening (DeHayes *et al.*, 1997). These were not apparent in whole-needle Ca levels nor in one-year-old foliage. Initial freezing temperatures were experienced in mid-November.

role in cascade systems equips Ca to activate a wide variety of enzyme systems. Ca-activated enzyme synthesis is also known to play an important role in the formation of two protective compounds that help the plant seal off wound areas: callose and lignin (Dixon *et al.*, 1994).

The signaling and response system by which callose is formed is now well known (Kaus, 1987). It is one of a wide variety of enzymatic systems in which calmodulin plays a central role as a Ca-mediated signal (Roberts & Harmon, 1992). Plant cells respond rapidly to cellular injury to form callose, a carbohydrate polymer of α -1-3 glucans that serves to plug damage and/or strengthen stressed cell walls and membranes. The Ca-activated enzyme which stimulates callose deposition is α -1-3 glucan synthase, and the signal for callose formation, which may start as rapidly as 10 min after a wound signal, can be produced by cell wall fragments generated by injury or by significant disruption in membrane permeability (Kaus, 1987). A key component in activation of callose deposition on cell walls in response to these fragments is a shift in membrane permeability, and a release of Ca. Removal of bound Ca from membranes also apparently reduces their capacity to form callose. Loss of membrane integrity is in itself a potentially important factor in the virulence of many pathogens for which containment of nutrient flow from the host to the pathogen is an essential part of defense (Hancock & Huisman, 1981). A demonstrable decrease in cellulose (α -1-4-glucan synthetase) activity and a rapid increase in callase (α -1-3 synthetase) in response to wounding (Delmer *et al.*, 1985) suggests that plants are equipped rapidly to shift their biosynthetic machinery from growth to structural defense when challenged.

Lignin is another structural defense agent, which can be activated in response to wounding and infection (Dixon *et al.*, 1994). Increased lignin synthesis has been shown to have both local and systemic control elements in cucumber (*Cucumis sativa*) plants (Dean & Kuc, 1987). Thus, the initial wound signal can produce enhanced lignin synthesis potential in leaves not initially infected by the pathogen. Increased lignin synthesis in tree stems is a prominent feature of the response of both balsam fir (*Abies balsamea*) and Fraser fir to attack by the Balsam woolly adelgid (*Adelgis balsamea*), an aphid-like sucking insect that has caused significant mortality to these species in eastern North America in the past three decades (Timmel, 1986).

Ca appears to play a role in lignin synthesis in both normal tree-growth processes (Eklund & Eliasson, 1990) and lignin increases in response to wounding. Increases in peroxidase, which stimulates lignin synthesis, have been associated with the systemic effects of infection in cucumber leaves (Dean & Kuc, 1987). Rapid increases in peroxidase activity have also been suggested as part of a Ca-moderated response to the generation of action potentials in wounded plants (Panel *et al.*, 1985).

In summary, Ca plays an essential role in all of the basic categories of plant defense against disease: maintenance of membrane integrity; signaling multiple pathways of defense through enzyme activation; release of phytoalexin, an antimicrobial agent; repair and reinforcement of damaged membranes and cell walls; and synthesis of structural barriers.

(e) *Cold tolerance.* The importance of cytoplasmic Ca levels in maintenance of plant membrane permeability led Minorsky (1985) to postulate that Ca played an important role in protecting plants from chilling injury. Maintenance of membrane integrity

in promoting cold tolerance is, in some respects, analogous to the protective role of Ca in defense against plant disease (Davies, 1987a). Membrane regulation of water extrusion from the cytoplasm during cold hardening, as well as resistance to cellular dehydration during the formation of extracellular ice crystals, is involved in freezing resistance (Levitt, 1978; Guy, 1990).

Interest in the physiology of winter damage to red spruce (*Picea rubens*) in the north-eastern USA has been an important aspect of research examining declines in growth of this species at high-elevation sites during the past three decades (Johnson *et al.*, 1988; Cook & Johnson, 1989; DeHayes, 1992). It is now apparent from analyses of seasonal changes in membrane-associated Ca in red spruce foliage (DeHayes *et al.*, 1997) that increases in such Ca are related to increased cold hardening. Temperature fluctuations were found to regulate the membrane-associated Ca and the subsequent dehardening-hardening cycle in red spruce as shown in Fig. 4. This study documented an important aspect of Ca physiology of plant organs – that a significant change in a very small, but physiologically active compartment can be sufficient to alter physiological performance of that organ in the absence of demonstrable changes in overall tissue levels.

(f) *Stomatal regulation.* Ca plays a role in control of leaf gas exchange through its regulation of stomatal guard cell turgor relations and stomatal opening and closing (McAinsh *et al.*, 1996). Stomatal closure is promoted by high concentrations of cytosolic free Ca in guard cells, which can in turn be stimulated by several types of environmental stimuli, including abscisic acid, increased CO₂ and oxidative stresses (McAinsh *et al.*, 1997). Although the specific mechanisms of Ca-induced closure are not known, oscillations in cytoplasmic Ca concentrations are thought to be related to apoplastic supply rates and to provide stimulus-specific signatures that enable plants to differentiate among stimuli (McAinsh *et al.*, 1996).

Viewed from a whole-plant perspective, linkages between fluctuations in stomatal guard cell function and Ca concentrations in the transpiration stream could potentially provide a sensitive indicator of the balance between Ca (and water) uptake and use along the transport pathway from roots to shoots. Operating in conjunction with plant water potential, Ca concentrations in the transpiration stream would provide a means of influencing water-use efficiency. Rapid growth, and associated depletion of Ca, may thereby provide a signal of a physiological 'need' for open stomates driven by Ca depletion at growth centers along the transpiration pathway; slowed growth would lead to increased Ca concentrations in the transpiration stream. Such a strategy would facilitate stomatal closure, and improved nutrient and water-use efficiency at slower growth rates.

The capacity of plants to maintain adequate transpiration rates in highly Ca-enriched environments would be challenged by such a strategy. However, calcicoles can sequester incoming Ca as Ca-oxalate in epidermal trichomes, and thus avoid high Ca concentrations in guard cells and stomatal closure (DeSilva *et al.*, 1996). Accumulation of Ca-oxalate crystals in the woody tissues of trees in arid environments may be indicative of a similar sequestration strategy (Gourlay, 1995) that helps them adapt to the excess supply potential of their growing environment.

III. CALCIUM UPTAKE AND DISTRIBUTION AT THE WHOLE-PLANT LEVEL

Bangerth (1979) reviewed a wide range of studies that indicate that Ca supply patterns in plants might be analogous to the source-sink concept by which carbohydrates are distributed along gradients from points of production to points of greatest use (Waring & Patrick, 1975). These Ca gradients appear to be primarily driven by relative rates of water movement to transpiring organs and relative rates of Ca use along the transport pathway. Thus, rapidly growing stems, twigs, or foliage act as competing sinks for directional Ca flow and use (Clarkson, 1984). Together with Ca uptake rate from the soil, the relative activities and distribution of these utilization centers are important in determining relative availability of Ca to storage organs and their resistance to many types of disease. Reduced availability of soil water can also significantly increase the severity of Ca-deficiency disorders in storage organs (Bangerth, 1979; Ferguson, 1984), an apparent consequence of linkages of Ca supply with the transpiration stream.

1. Uptake at the root-soil interface

Ca uptake into roots occurs principally by passive movement in the mass flow of soil water driven by the transpiration stream (Bangerth, 1979). Thus, root 'activity' in obtaining Ca is indirectly keyed to carbon assimilation in the canopy through concurrent regulation of fluxes of water and carbon through stomates. The concentrations of Ca in the soil solution are controlled by soil chemistry and diffusion along concentration gradients. Critical features of Ca supply capacity include the soil cation exchange capacity, the proportional representation of Ca in the exchangeable base cation pool, the rate at which mineralization of soil organic matter and primary minerals release soluble Ca, and the pH of the soil solution (Ulrich & Matzner, 1986). Total cation concentration is also balanced and controlled by levels of mineral acid anions in the soil solution (Robarge & Johnson, 1992).

It is important to recognize the nonlinearity of the effects of soil pH on Ca availability in soil solutions (Reuss & Johnson, 1986). At pH levels ≥ 5.0 ,

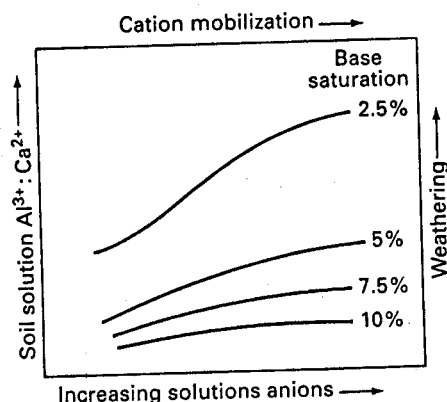


Fig. 5. Ca availability in soil solutions is strongly influenced by soil weathering, cation-exchange capacity of soils, base saturation and inputs of strong acid anions, which mobilize exchangeable Al more rapidly than Ca. At lower base-saturation levels, large changes in relative availability of Al and Ca are produced by relatively small changes in ionic strength. At Ca:Al ratios ≤ 1.0 Ca availability to plant roots is further reduced by chemical interference of Al with Ca uptake.

increasing acidity is associated with both increased mineral weathering and increased availability of Ca within the soil solution. This is effected by replacement of Ca on soil cation-exchange sites by H^+ or other acidic cations and release of Ca to the soil solution, from which it may be absorbed by roots or lost by leaching. Both natural and human influences that acidify soils will make a relatively larger proportion of the soil-exchangeable Ca available in the soil solution (Ulrich & Matzner, 1986). As soil pH drops below 5.0, Al^{3+} is mobilized more rapidly than Ca^{2+} , because release of cations is proportional to charge, resulting in a greater relative representation of Al in the resulting soil solution (Reuss & Johnson, 1986). In addition, as the pH drops below 4.5, significant stores of Ca-oxalate, which in some soils can represent half of the exchangeable pools in the forest floor, can become rapidly dissolved (Cromack *et al.*, 1979). The mobilization of Ca by anion loading is not tied to the natural annual cycles of temperature and associated processes of decomposition and root growth that help match nutrient uptake with nutrient availability. For this reason, stochastic deposition patterns release 'excess' cations that may be lost from the system during much of the annual cycle. Over time this will result in loss of base saturation of exchange sites, unless weathering and decomposition re-supply the system at rates equivalent to losses to uptake and leaching.

At very low soil-solution pH, Ca mobilization is reduced, Al becomes the dominant cation, and resulting Ca:Al ratios decrease. Mineral-acid anions are particularly important in determining cation concentrations at this stage because, as anion concentrations increase, a greater proportion of exchangeable cations, including Ca, but especially Al, are released from exchange sites within the soil complex.

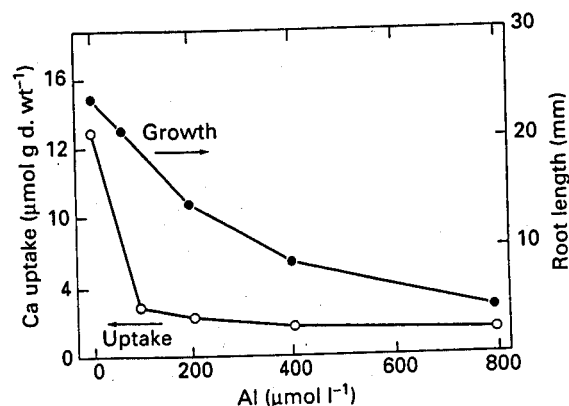


Fig. 6. Al concentrations affecting Ca uptake and root elongation for Norway spruce seedlings in solution culture indicate a relatively greater effect of Al on Ca uptake than on growth at lower Al concentrations (Godbold & Kettner, 1991; Godbold *et al.*, 1988).

The interplay of soil weathering, percentage base saturation, and anion-induced mobilization in influencing Ca:Al ratios in soils is depicted in Fig. 5. Ca availability for root uptake decreases even more rapidly below soil pH 4.0, because Al, a strong competitor with Ca with a large soil reservoir, is released even more rapidly than Ca and interferes with Ca uptake and root growth. Studies with tree seedlings in nutrient culture indicate that at soil solution Ca:Al ratios below 1.0, Al interferes with Ca uptake by roots (Rost-Siebert, 1983; Schroeder *et al.*, 1988). Patterns of Ca uptake and growth in nutrient solutions (Fig. 6) indicate that interference with Ca uptake occurs at lower Al concentrations than are required for direct inhibition of root growth (Godbold *et al.*, 1988; Godbold & Kaettner, 1991).

Bangerth (1979) described typical Ca concentrations at the root-soil interface as 0.1–1 mM compared to soil-solution values in the range of 3.4–14 mM. However, in forest systems, particularly those in later successional stages or in industrialized regions, soil-solution Ca values may be much lower and associated with low Ca:Al ratios and interference from Al. Johnson *et al.* (1991), for example, found typical A-horizon soil solution Ca values to range between 10 and 150 μM and Ca:Al ratios to average approximately 0.4 for a high elevation southern Appalachian (USA) red spruce forest. Values recorded at a comparable depth in a beech (*Fagus sylvatica*) forest in Germany were in the range 10–600 μM Ca (Matzner & Prenzel, 1992).

The typical pathway for uptake of mineral elements involves initial movement into the free space of the root apoplast, and subsequent basipetal movement through apoplastic pathways. This pattern of movement through the cell walls avoids trans-membrane passage and the toxicity problems that contact with the cytoplasm would entail (Haynes, 1980). Thus, Ca uptake is confined to the unsubsized fine root tips that have no casparian

strip, which would force a symplastic detour. In addition, because xylem movement of Ca is unidirectional and passive, fine roots cannot be resupplied with Ca from basipetal tissues and are particularly susceptible to low Ca supply from the soil (Bangerth, 1979).

The status and the cation-exchange capacity of the apoplast is particularly important for Ca uptake, because Ca must move entirely through the apoplast to the conducting xylem. In the apoplast, the cation-exchange capacity of the cell wall is influenced by the relative concentrations of cellulose, hemicellulose and lignin, constituents that can vary significantly between plant species and can have an important influence on adsorption of specific ions. Below pH 4.0, uptake of both Ca and Mg by fine roots of Norway spruce seedlings is blocked by competing ions in hydroponic cultures in the order of $Al > H > Fe > Mn$ (Stienen & Bauch, 1988). Interference with and/or replacement of the less tightly bound Ca is a part of this effect. Schroeder *et al.*, (1988) found that the combination of pH 4.5 and increased Al:Ca ratios reduced Ca binding in root cell walls by 83%. The low Ca binding (-80%) found in cell walls of trees from a field site acidified by atmospheric deposition (soil pH 3.0) was partially reversible by subsequently adding pH 4.7 solution, but entailed a loss of 40% of the original exchange capacity. Thus, residual effects on root uptake of Ca can be produced by prior exposure to high Al concentrations.

Although coping with low Ca supply is only one of several stresses that plants growing in very acidic environments must face, interspecific differences in the cation-exchange capacity of root cell walls may provide some adaptive advantage in Ca uptake. The cation-exchange capacity of root cell walls has been found to be reduced in acidophilous herbs (Blamey *et al.*, 1990; Koedam *et al.*, 1992). The suggested gains from such a strategy were attributed to reducing Al displacement of Ca from cell walls (Koedam *et al.*, 1992). This adaptation would appear to represent a gain if cell-wall Ca involved in tissue structural integrity were protected at non-exchangeable sites, or if low retention of Ca in the root walls facilitated rapid movement to shoots.

2. Transport and exchange in stems

Ca is found in greater quantities than any other inorganic element in plants, and, as previously noted, its influx to the xylem is a function of transpiration flow (Marschner, 1995). Thus, like water supply, the flow of Ca is closely linked to the functional leaf area maintained through the conducting sapwood cross-sectional area (Rogers & Hinckley, 1979; Whitehead & Jarvis, 1981; Ryan, 1989). Whereas transpiration determines the total amount of Ca that enters the tree, annual growth rates regulate the Ca that is incorporated into woody tissues.

Although Ca moves as a part of the transpiration stream, the pathway is no longer considered to be a direct one. Rather Ca is now considered to move as a part of an ion-exchange complex, with adsorption and desorption occurring from active exchange sites within the cell walls of the xylem sapwood along the way (Ferguson & Bollard, 1976; Hanger, 1979). The ion-exchanging stem wood functions as a column in equilibrium with the transpiration stream (Momoshima & Bondietti, 1990). On sites with adequate Ca supply, growth rate appears to be inversely related to potential Ca exchange activity in the xylem. This occurs because increased growth is associated with wider, but reduced numbers of, annual rings in the functional sapwood area. In slower growing trees, with smaller annual growth rings, more rings and a larger surface area of cell wall surfaces are included in the conducting cross-section, thereby increasing the potential influences of cell wall exchange on Ca supply rates. Therefore, a slower growing tree will retain sap flow within a particular annual ring longer than a faster-growing tree with sapwood that has less rings involved. Increased surface-area contact within the sapwood increases the opportunity for exchange reactions between Ca in the sap and the cell walls (Guyette & Cutter, 1995). Species differ in this respect because of differences in xylem structure that result in systematic variations in efficiency of water transport through the stem. The role of reduced Ca supply in the production of smaller rings is an interesting issue, and whether it represents a consequence of slow growth or an adaptation to nutrient supply limitations that limit growth is unknown.

The cation exchange capacity of red spruce xylem has been examined under laboratory conditions and found to be very sensitive to both sap pH and radial distance from the pith (Momoshima & Bondietti, 1990). The Ca content of the xylem was estimated to occupy 60–80% of the cation binding sites. Increased sap acidity resulted in displacement of Ca from cell wall exchange sites. In addition, age/diameter-related decreases in binding capacity result in a natural pattern of decreasing Ca retention with tree age (Momoshima & Bondietti, 1990; Shortle *et al.* 1995). This is apparently attributable to a decrease of the cellulose:lignin ratio accompanied by increased crystallinity of cellulose in older wood (Erickson & Arima, 1974; Wellwood *et al.*, 1974). The relationship of adsorbed Ca in stemwood to sap pH and Ca concentration is thought to represent an equilibrium reaction involving feedbacks between sap pH, sapwood binding status, and concentrations of Ca and competing cations. For example, either an increase in sap acidity by 0.5 pH units at constant Ca concentrations or an 80% drop in sap Ca concentrations at constant pH was found to reduce cell wall binding of Ca by about 25% (Momoshima & Bondietti, 1990).

The cell wall surface contains a high number of negative binding sites provided by lignin and cellulose. Total Ca exchange capacity of individual tracheids depends on the inner surface area, as well as the ultrastructural and chemical composition of its cell walls. The cation exchange capacity per cm² vessel surface has been calculated at the rather high value of 10⁻⁷ mol equivalent which led Van de Geijn & Petit (1979) to hypothesize that cations must penetrate into the cell wall. For a 2 mm-thick cell wall, Kuhn *et al.* (1997) calculated cation exchange capacity to be 300–500 mol m⁻³. These authors were also able to demonstrate in a LAMMA/SIMS microprobe study that Ca is evenly distributed within the cell wall, which means that Ca may access the entire donan free space of the apoplast.

These studies raise a series of questions regarding the significance of changes in cell wall Ca measured at near-ground level:

- What fraction of the total available Ca in cell walls of both roots and stems is actually exchangeable under field conditions?
- How do these changes affect the level and timing of Ca supplied to the growing centers further up the stem?
- What are the consequences of changes in cell wall Ca on the structural integrity of cell walls themselves?

Isotopic analysis of cation exchange by Norway spruce roots (Kuhn *et al.*, 1995) suggests that the exchangeable fraction in cell walls of fine mycorrhizal roots, at least, is quite high, approaching 100%. In addition, occupancy of binding sites in the cell wall by alternate cations, such as Al, was found actually to facilitate movement of Ca through the xylem to apical growth centers. This resulted from more basipetal anionic binding sites in the stem being blocked. Thus, although net uptake was reduced by lower pH and the presence of Al in solution, a higher fraction of Ca absorbed by roots moved up the stem past occupied binding sites along the path from roots to shoots. This raises an interesting question relative to the potential role of the exchangeable Ca in the cell wall as a reservoir for Ca supply to acropetal growing centers. Displacement of 'bound' Ca by Al under intermittent acidic pulses related to soil warming events, or more gradual increases of either natural or anthropogenic origin, might serve to increase stem Ca supplies to the upper canopy in forest trees. This would result from displacing bound Ca in xylem wood and by reduced adsorption of Ca in the sap stream at sites now occupied by Al or other acidic cations.

3. Ca exchange by foliage

Plant uptake of Ca from the soil is partially offset by relatively high Ca losses from foliar leaching by

Table 1. Net canopy fluxes of K, Ca, and Mg and relationships to total base cation exchange from forested sites in the IFS study (Ragsdale *et al.* 1992)

	Flux (Sd)	
	Eq ha ⁻¹ yr ⁻¹	% of total base cations
K	199 (97)	51 (11)
Ca	113 (48)	21 (9)
Mg	68 (33)	13 (6)

Data are means (+1 SD) from 12 sites.

incident rainfall. Among the cations exchanged by a deciduous canopy in east Tennessee (USA), Ca-leaching rates exceeded those for Mg by approximately 50% and comprised from 40% (yellow poplar, *Liriodendron tulipifera*) to 75% (oak, *Quercus* spp.) of the total cations leached (Johnson *et al.*, 1985). In general, total Ca losses are dependent on total leaf area and are higher for mature forests, late successional species and evergreen canopy types. A comparison of net canopy exchange of Ca from 12 forests examined in the Integrated Forest Study (IFS; Johnson & Lindberg, 1992), as shown in Table 1, indicates that annual leaching rates ranged from > 50 to > 200 eq Ca ha⁻¹ yr⁻¹ (Ragsdale *et al.*, 1992). The significance of foliar Ca exchange at these levels is potentially high, both in terms of the total annual Ca accumulation by forests and in terms of standing pools in foliage. Compared to net annual increment, average foliar Ca losses of 113 kg ha⁻¹ (Table 1) exceeded net annual forest increment for some Ca-depleted forest systems and amounted to over 50% of net annual uptake for approximately 30% of the forests compared in IFS (Johnson & Lindberg, 1992; Fig. 7). Compared to standing foliar Ca pools, leaching rates for a mature forest canopy in Tennessee equated to approximately 20% of the annual foliar Ca pool for yellow poplar but only 12% of that pool for oak (Johnson *et al.*, 1992). These losses compare favorably to the 4–13% leaching losses for an early successional forest in nearby North Carolina (Potter *et al.*, 1991).

It may be argued that Ca leached from foliage is not truly lost from the system because subsequent root uptake and cycling occur from the soil; however, it is important to consider the origin of leached Ca. Detailed chemical Ca budgets show that a significant fraction of leached Ca is derived from internal sources. Microscopic analysis of internal Ca distribution of conifer needles leached by acidic rainfall indicates that depletion of Ca occurs from apoplastic reserves (Fink, 1991). Measurements of changes in droplet chemistry on prewashed dogwood (*Cornus florida*) leaf surfaces indicate that Ca efflux from the dogwood leaf interior was rapid, occurring within minutes of contact, and 32–98% of the droplet H⁺

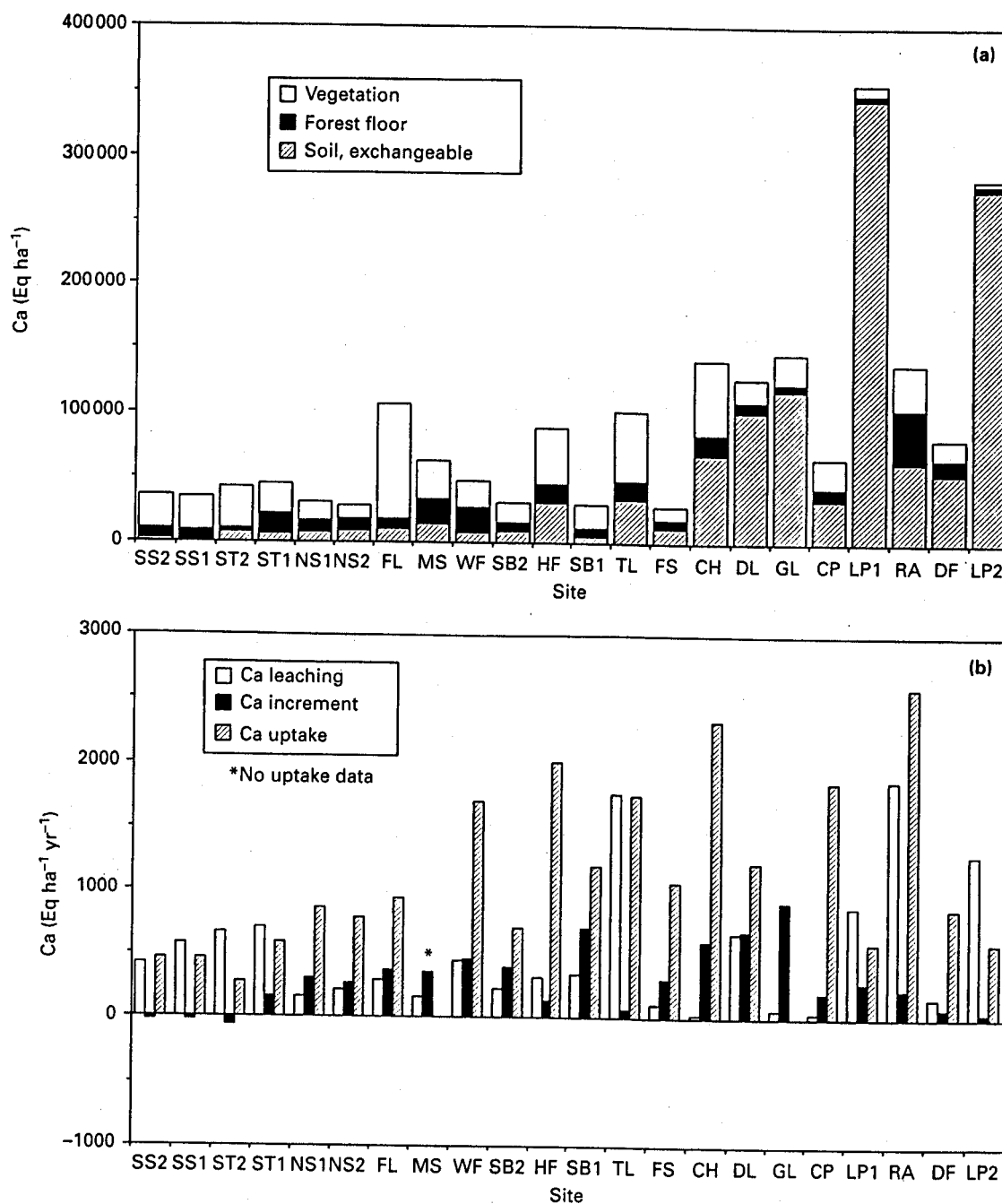


Fig. 7. Comparison of Ca pools and fluxes for diverse forest types from the Integrated Forest Study (Johnson, 1992). (a) Pools in vegetation and in the forest floor dominate soil-available Ca for many forest types. (b) Leaching losses vary significantly in magnitude and importance relative to Ca increment across these IFS sites (Johnson, 1992). Forest-site types discussed in this paper include high-elevation red spruce in the Southern Appalachian mountains (SS and ST) and at Whiteface Mountain, New York (WF). Other forests represented include Norway spruce (NS) in Norway, southern hardwoods (CH at Coweeta, North Carolina), northern hardwoods (HF in New York) and southern loblolly pine (DL, GL and LP).

was exchanged for Ca^{2+} (Willey & Hackney, 1991). Loss of leaf Ca in a single 1-cm rain event at pH 4.0 was sufficient produce a Ca concentration of 18–24 mmol in foliar leachate and a loss of 1% of total foliar Ca. In cell systems in which cytoplasmic concentrations are in the range 10^{-6} – 10^{-8} M (Bush, 1995), shifts of 1.8 to 2.4×10^{-5} M Ca would be anticipated to alter metabolic rates and induce shifts in the homeostasis of cells.

Thus, although Ca loss from individual rain events may be relative small relative to total leaf Ca (Mitterhuber *et al.*, 1989), the cumulative effect of these losses over a typical growing season may be large if considered from the perspectives of either the cumulative loss over an entire season or the episodic loss of Ca concentrations that are large relative to the micromolar quantities involved in intracellular Ca signaling just discussed. This is indicated by nu-

merous studies that now demonstrate the adverse physiological effects of increased foliar leaching in rainfall acidified with strong acids (Schulze, 1989; McLaughlin & Kohut, 1992).

IV. ECOSYSTEM PROCESSES AND CALCIUM SUPPLY

Processes that regulate Ca availability and influence its effects on plant production over longer time periods and larger spatial scales are of interest from the perspectives of ecosystem function and regulation. Our interest here is both in the changes that occur naturally over space and time, but also in evidence that these changes significantly influence plant and community structure and function.

1. Plant succession and soil acidification

Plant succession depends on well recognized inter-relationships between the growth habits and resource requirements of competing vegetation and changes in the capacity of the site to supply required nutrients and water, and to distribute solar radiation among components of the plant community over time. Modification of forest soils by the chronosequence of vegetation types that leads from pioneering species to a climax forest community is an important part of forest successional processes. Over long time frames, it will lead to major changes in availability of all of the plant nutrients, but particularly Ca and N.

Ulrich (1983, 1984) describes a typical chronosequence of post-glacial primary succession that leads from a base-rich, N-poor soil condition to a forest community in which increasing amounts of cations and N are incorporated into vegetation, and into the soil organic matter that helps buffer the system against nutrient deficiency. This whole process, which may take many hundreds of years fully to develop, is centered around the chemical requirement to balance the reducing power of photosynthesis and organic acids it produces with neutralizing influences of cations removed from the soil and incorporated into phytomass. It leads ultimately to a more acidic forest soil, reduced concentrations of base cations, particularly soil Ca, and accumulation of these cations in woody biomass, particularly foliage and branches, and in the organic humus layer (Ulrich & Matzner, 1986).

The acids produced during the early aggrading stage of forest development are initially weak carbonic acids from organic matter decomposition and root respiration. However, as organic matter and associated N accumulate over time, soil pH levels are reduced by nitric and organic acid production associated with accumulation of humus in the upper soil horizon. Thus, forest growth itself is an acidifying process that leads to three important chemical processes that reduce the availability of Ca

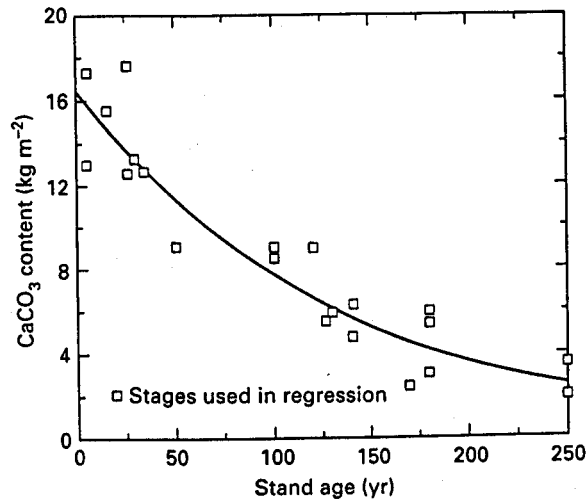


Fig. 8. Loss and/or organic sequestration of Ca from soils is an important component of natural succession. In this example, which began with Ca-rich glacially derived substrate soil, Ca levels both affected and were affected by changing community composition and nutrient-use patterns (Marion *et al.*, 1993a).

for plant uptake over time. These include: incorporation of Ca into the woody biomass, where it is temporarily unavailable to the system; loss of available Ca from the soil through increased leaching as increasing amounts of exchangeable Ca from soil reserves are replaced by H^+ in the soil-exchange complex; and reduced availability of Ca to the roots attributable to competitive interference from Al and other acidic cations that increase in the soil solution as pH levels drop to values < 5.0 . Soil acidification is not a process restricted to industrial regions with high atmospheric deposition of strong acids, but can significantly influence Ca availability in remote forest systems as well.

The decreasing trend of soil Ca that occurs during the earlier stages of the developmental chronosequence described by Ulrich (1983) is well illustrated by soil development occurring with sequential life stages during a 250-yr primary successional sequence along the Tanana River in Alaska (Marion, 1993; Fig. 8). These soils lost approximately 75% of the original Ca content over 250 yr from internal acid production, which was calculated to have added 10 keq ha^{-1} to the system each year. Although still alkaline at the time of the study, the soil profile had acidified by 2–3 pH units (pH 8.4 to pH 5.4–5.6) in the upper 10 cm at the oldest (white spruce, *Picea glauca*) successional stage (Marion *et al.*, 1993).

With time, natural successional processes, combined with climate, lead to the redistribution of organic matter and associated base cations from deeper profiles to an N-enriched organic surface horizon. Strong mineral and organic acids generated in this layer leach the mineral soil below, and associated cation losses and uptake by vegetation may greatly exceed buffering and resupply by

type. In this study, root growth into implanted vermiculite cylinders, both with and without nutrient addition, was several times higher on a Ca-deficient Amazonian forest soil than in forests where N, P, or K were primary limiting nutrients.

The high effectiveness of even relatively small increments in Ca on overall root-growth responses raises an important point about evaluation of fertilizer responses in Ca-limiting environments – the addition of N with an associated strong anion will increase Ca availability if soils have adequate base saturation to permit Ca mobilization into the soil solution (Fig. 5). Improved root growth and overall plant nutrition by these interactions could make separation of primary and secondary nutrient effects of N addition difficult under these conditions.

Experiments involving addition of more Ca with seedlings and saplings confirm the potential significance of increased Ca supply for tropical forest species. These include experiments with acid-leached soils from a tropical rain forest in Singapore (Burlsem *et al.*, 1995) and physiological studies with 13 woody species from an oligotrophic Amazonian rain forest (Reich *et al.*, 1995). These experiments, together with the documentation of Ca deficiency of mature Terra Firme Amazonian forests by Cuevas and Medina (1988), indicate that Ca can be the primary nutrient limiting tree growth in cation-depleted tropical settings. Particularly relevant are physiological studies that have explored both changes in relative availability of Ca, N and P and their physiological effects for species spanning early to late successional stages in the Amazonian rain forest (Reich *et al.*, 1995). Foliar Ca, P and N were found to be highest in early successional species, although only Ca continued to decline through the later successional stages. Although photosynthetic rates were correlated with N and P in early successional species, at the lower levels of Ca, N and P occurring with later successional species, leaf photosynthetic rates were strongly and linearly correlated with leaf Ca concentrations ($R^2 = 0.80$) and not with leaf concentrations of N or P (Reich *et al.*, 1995).

Other evidence that Ca is an important component of the nutrient-deficiency complex that alters growth patterns of tropical rain forests includes:

- The absence of typical structural modifications indicative of nutrient limitations on N-poor sites that have moderate Ca levels (Scott *et al.*, 1992).
- The presence of stunting patterns at other sites that have adequate N levels (Bruijnzeel *et al.*, 1993).
- The nature of nutrient-conserving adaptations that occur in these forests.

Clearly, additional field experimentation is needed to evaluate both functional and structural responses of tropical forests to increases in Ca supply.

2. Plant adaptations to nutrient deficiency

One measure of the significance of nutrient limitations to plant development and function is the diversity of plant morphological and physiological adaptations to conditions that limit nutrient supply. We examine here first the spectrum of adaptations that influences plant nutrient acquisition and conservation, and subsequently, the potential importance of Ca deficiency as a driver of such adaptations. We consider the adaptations and their linkages to Ca supply as one measure of the strength of our original hypothesis – that Ca limitations can significantly influence forest structure.

(a) *Morphological adaptations.* Chapin (1980) has reviewed a wide range of native plant 'strategies' for reducing nutrient stress, and these are summarized briefly here as a basis for further hypothesis testing. Examples cited by Chapin were developed mainly from studies involving N and P limitations on native herbaceous plants, but many of the same principles apply with cations as well, and where they do not, the contrasts can be useful. Plants in general shift resources between roots and shoots depending on where the resources in most growth-limiting supply are acquired. As a consequence, growth under constraints in resource availability is typically shifted towards the organs involved in acquisition of the resource most limiting overall plant growth (i.e. the site of maximum availability of the most limiting resource).

The influences of nutrient stress and low light on shifting root:shoot ratios are classical examples of adaptations that result in a shift in carbon allocation to overcome low nutrients (higher R:S) or lower light (higher S:R). Although these adjustments are possible to varying degrees within the physiological plasticity of individual plants and species, there are also interspecific differences that appear in the successional sequence that leads from pioneering species, where light and nutrients are generally more abundant, to late successional species where these resources become more limiting. Thus, both nutrient and shade stress develop in parallel over time during forest succession.

Chapin (1980) emphasized the importance of slow growth rate as an adaptive strategy with both physiological and phenological consequences. Slower growth rates, and lower nutrient demand, equip late successional species to survive at low nutrient supply rates. This strategy may also facilitate resumption of growth following episodic nutrient stress periods, which can terminate seasonal growth of faster-growing species. Prominent among the nutrient-conserving strategies discussed by Chapin (1980) are adaptations involving leaf longevity and structure. The general pattern with later successional species is toward increased leaf longevity and sclerophylly, both strategies that reduce

Table 2. Percentages of forest stands with $NUE \geq 25\%$ above the minimum value at $>50\%$ of the observed range of litter content levels for each forest type

Element	Forest type		
	Tropical % (sample No.)	Deciduous % (sample No.)	Conifer % (sample No.)
Ca	71 (31)	42 (24)	69 (16)
N	33 (28)	58 (26)	50 (32)
P	31 (31)	39 (23)	44 (16)

Data were derived from Vitousek (1982). Increased responsiveness of NUE to reduced Ca relative to N and P suggests that Ca becomes limiting at higher relative nutrient contents than N or P for some forest types.

nutrients. For example, the ranges in ratios of maximum to minimum nutrient concentrations in foliage of Amazonian forest species were 1:65, 1:12, 1:10, 1:5 and 1:4 for Ca, Mg, K, P and N, respectively (Reich *et al.*, 1995). These data suggest that either Ca content is not very important, and hence that plants can exist in a wide variety of conditions with little regard for Ca concentration or, alternatively, that Ca is very important and that plants have adapted differential Ca uptake and utilization strategies to address these variations.

Vitousek (1982) developed the concept of nutrient-use efficiency (NUE) of leaf litter as a measure of the capacity of diverse forest types to increase foliar biomass production per unit of nutrient mass under conditions where total nutrient uptake and return in litter is reduced. He concluded from increases in NUE at low nutrient-supply rates that increased physiological efficiency of utilization of N, P and Ca occurred as a physiological adjustment to low nutrient supply. These concepts are difficult to apply to Ca in a way comparable to N or P, because both N and P are retranslocated before leaf senescence. However, our analysis of the changes in apparent NUE among nutrients for three forest types, shown in Table 2, suggest that, for some forest types, calculated NUE responds more readily to reduced Ca supply, even when expressed at the whole-leaf level, than to either N or P. This is supported by the data of Cuevas and Medina (1986), who noted that Amazonian forests of Tierra Firme, which are relatively high in N and very low in Ca, have leaf litter with high Ca NUE values.

In the Vitousek (1982) analysis, two data points stood apart as outliers, both having unusually low NUE and low total Ca return in litterfall. Both were from the heavily polluted temperate coniferous forest in Solling, Germany (Cole & Rapp, 1981) for which element fluxes, particularly N, S, Ca and Al, have been significantly altered by combinations of natural and anthropogenic acidity (Ulrich & Matzner, 1986). The very low Ca uptake and return in litter in this acidified forest ecosystem suggests that both uptake and use of Ca were reduced at these sites. This is

supported by substantial mechanistic evidence of the role of anthropogenic inputs of acidity in amplifying natural acidification processes and biogeochemical cycles that regulate availability of Ca and other base cations for plant uptake (Ulrich & Matzner, 1986; Robarge & Johnson, 1992). The causes and implications of these limitations for forest processes provide further insights into Ca regulation of ecosystem processes at both whole-tree and community scales.

V. PLANT AND ECOSYSTEM RESPONSES TO HUMAN ALTERATIONS IN CALCIUM SUPPLY

The appearance in recent decades of widespread and diverse symptoms of physiological stress on several forest tree species in industrialized regions of the USA and Western Europe has been extensively reviewed and debated with respect to both primary and secondary causes and long-term significance to forest health (Rehfuess, 1981; Schutt & Cowling, 1985; McLaughlin, 1985; Prinz, 1987; Pitelka & Raynal, 1989; and Rehfuess, 1991). In the case of European forests, increased forest growth observed in many areas in the past two decades raises interesting questions about the causes and significance of widespread foliar symptoms of stress clearly apparent in many areas of Central Europe in the late 1970s and early 1980s (Sterba, 1996). Evaluation of this apparent anomaly necessitates considering three factors that are important in evaluating plant responses to any stress: mechanisms of effect, changes in stress level over time and interacting environmental variables. Here, we address these issues in the context of the role of base cation supply in areas where responses have been documented, and the effects of time, location and atmospheric N deposition on forest physiology and forest nutrient cycles.

1. Increased atmospheric inputs of acidity

Acidification of forests by natural processes of cation sequestration and internal acid production has been augmented in recent decades over widespread areas

high-elevation sites in the Great Smoky Mountains, for example, very high Ca deposition levels, coupled with high leaching rates and low Ca uptake by vegetation, are apparently leading to net accumulation of Ca even in the presence of high leaching rates (Johnson, 1992). Here apparent low uptake of Ca by vegetation may constitute a form of self-regulation that has potentially adverse effects on forest physiological function.

(b) *Inhibition of calcium uptake and effects on root function.* The elucidation of the reaction kinetics for Al and Ca mobilization in soils (Reuss & Johnson, 1986) has led to the realization that large and rapid changes in soil-solution chemistry are possible as a result of strong anion inputs from the atmosphere (Robarge & Johnson, 1992). The non-linearity of changes in Ca and Al in soil solutions at lower soil pH values and low base saturation means that rather small changes in soil acidity or, alternatively, small changes in ionic strength of the soil solution can significantly shift the Ca:Al ratio (Reuss & Johnson, 1986; Fig. 5). It is important to note that this response requires only inputs of strong anions such as SO_4 and NO_3 , not changes in soil pH *per se*.

Al toxicity has been an important consideration in the efforts to evaluate the potential of acidic deposition to affect forest ecosystems for almost two decades (Ulrich *et al.*, 1980). Effects of Al on root structure and function can be direct at higher concentrations or indirect as a consequence of interference with Ca uptake or retention. Oleksyn *et al.* (1996) reported changes in organic chemistry, growth, structure and function of roots of Scots pine (*P. sylvestris*) seedlings at Al concentrations (0.5 mM) that can be found in soil at European sites with high levels of acidic deposition. Controlled studies of Ca uptake, already discussed, indicate the importance of Al and other acidic cations in blocking Ca uptake and/or replacing Ca from the apoplast of root cell walls. Significant disruption of Ca flow to shoots and effects on both shoot and root growth in hydroponic studies with Norway spruce have been demonstrated at a soil solution Ca:Al ratio of 0.67, well above the seasonal volume-weighted mean Ca:Al ratio (0.36) measured at a high-elevation Southern Appalachian site in the USA (Johnson *et al.*, 1991).

Reduced Ca:Al ratios in roots of red spruce in the USA (Shortle & Smith, 1988) and Norway spruce in Germany (Bauch, 1983) have been considered an important indicator of declining tree vigor and root function of those species. Low Ca:Al in fine root tips, in addition to inhibiting root growth, induces increased levels of a stress-related polyamine, putrescine, which can be detected in the foliage of even apparently healthy red spruce trees (Shortle *et al.*, 1997). A broader evaluation of the implications of changes in the Ca:Al ratio in both soil solutions and tree tissues (Cronan & Grigal, 1995) has

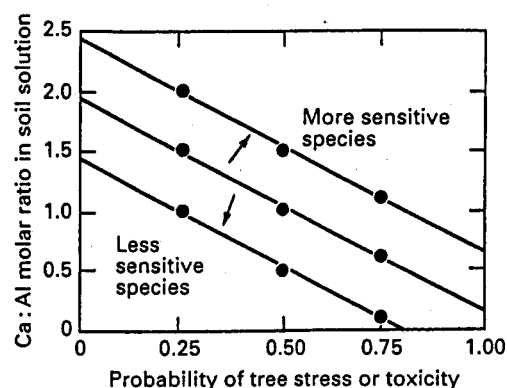


Fig. 10. A survey of forest responses to variations in the Ca:Al ratio in soil solutions has been used to develop generalized response surfaces that describe relative risks of impairment of forest processes by low Ca and high Al levels. Contrasts between mean, sensitive and more resistant species are noted (Cronan & Grigal, 1995).

indicated that this ratio may be a valuable indicator of changes in forest function. From a review of a wide range of studies, Cronan & Grigal (1995) classified the effects of decreasing Ca:Al ratios in the soil solution in terms of increasing relative risks to forest function (Fig. 10). The > 50% risk level in the mean response surface developed in Fig. 10 occurs at a Ca:Al ratio of 1.0, with sensitive species expected to respond negatively at about twice the Ca:Al concentration levels as resistant species.

Foliage typically experiences reduced function at higher Ca:Al ratios than roots. For example, the estimated 50% risk level associated with foliar Ca:Al values was > 12.5, 60 times higher than the threshold for 50% risk to fine roots (0.2) (Cronan & Grigal, 1995). This is more likely a result of reduced movement of the more-strongly adsorbed Al ion to shoots rather than higher sensitivity of foliage to Ca. Reduced Ca transport to foliage from roots can be caused by a combination of factors leading to either chemical interference with Ca uptake by Al, physiological effects on root integrity or function (Zhao, 1987), or reductions in root mass.

Root distribution within the soil profile is an important component of forest structure that also appears to be influenced by the combined effects of Ca and Al supply. The Ca:Al values of the mineral soil at acidic forest sites are typically quite low compared to the more base-rich organic soils just below the forest floor (Johnson *et al.*, 1991; Fig. 9), and this condition can limit rooting in the deeper mineral soils. Evidence that acidic deposition reduces the effective depth of root production within profiles of poorly buffered acidic soils comes from many different studies including: contrasts in fine-root distribution between healthy and declining Norway spruce stands in the field (Meyer *et al.*, 1988); contrasts in mature red spruce at field sites differing in cloud exposure and soil-solution Ca:Al levels (Joslin & Wolfe, 1992); examination of N-

this species (Robarge, 1989). These concentrations are substantially lower than the levels reported by McNulty *et al.* (1996) in response to soil-N additions of up to $31.4 \text{ kg N ha}^{-1} \text{ yr}^{-1}$, which raised foliar N concentrations from approx. 8 mg g^{-1} to 15 mg g^{-1} N. The study site in this case was a high-elevation site with relatively low previous N deposition levels ($5.4 \text{ kg ha}^{-1} \text{ yr}^{-1}$). The absence of high foliar N levels (Friedland *et al.*, 1988) in the presence of high N levels in soil solutions, combined with a relatively greater input of N from atmospheric sources, indicates that N uptake efficiency by roots, regardless of the form of N, may be rather low at these sites. Total foliar N uptake is also reduced by loss of tree basal area and reduced growth of remaining trees at many of these high-elevation spruce-fir sites (McLaughlin *et al.*, 1987). This raises the possibility that the efficiency of root uptake of N under these conditions may be reduced by N accumulation, Al mobilization, and associated effects on Ca uptake and growth of new absorptive roots. Rennenberg *et al.* (1998) found that NO_3 uptake in both spruce and beech forests could be completely inhibited by high N loads and attributed the effect to an accumulation of organic amino compounds translocated from shoots to roots. This accumulation would probably also be enhanced by processes that limit root growth and metabolite use.

Episodic fluctuations in soil solution Al levels in N-saturated forests are strongly related to SO_4 and NO_3 concentration in the upper soil horizons (Johnson *et al.*, 1991). As a consequence, Al concentrations and particularly Ca:Al ratios in the soil solutions of such forests can frequently reach levels (Al at $\geq 100 \text{ mm l}^{-1}$ and Ca:Al ≤ 1.0) that can affect root function (Fig. 11). Calculated Ca:Al ratios in the soil solution at this high elevation spruce site dropped to approx. 0.25 during high Al intervals and averaged 0.36 over the three-year study interval. Because periods of high Al and NO_3 concentration in soil solutions often occur together and at times when Ca:Al ratios are lowest, root uptake potential in these N-saturated soils would be out of phase with N availability. Thus, for N uptake from soils with low base saturation, the mobilization of Al by strong anion inputs means that biological availability of N would be lowest at the time that soil solution N levels are highest, thereby reducing N uptake and contributing to N accumulation. An additional, potentially important, component of reduced N uptake potential is reduced fine-root production and the shift in root distribution to shallower zones in the soil profile.

Yet, for many forests, current atmospheric sources of N may provide a fertilizer response. For example, under relatively low inputs of atmospheric N in northern Sweden, relatively young forests on more recently glaciated soils have a long history of positive growth responses to N fertilization and appear, in

general, to be far from N saturation (Binkley & Hogberg, 1997). Foliar Ca levels do not indicate obvious Ca deficiency. By contrast, forests along the southern coast, which receive the highest levels of N and S, are showing increasing signs of N saturation (Binkley & Hogberg, 1997). Fertilization experiments in southern Sweden provide evidence of positive growth responses to added Ca, which has been shown to increase the effectiveness of added N in stimulating growth (Nohrstedt *et al.*, 1993). Under these conditions, observed responses to N fertilization can still be positive even at base:Al ratios < 1.0 in the soil solution (Binkley & Hogberg, 1997). Foliar analyses from these studies indicate that Ca concentrations can be maintained at moderate levels ($\sim 3.0 \text{ mg g}^{-1}$) under Swedish growing conditions. Foliar Ca concentrations have also been shown to be reduced by N fertilization and significantly increased (38%) by irrigation with N-free liquid fertilizer containing modest Ca concentrations (218 kg ha^{-1}) (Nilsson & Wiklund, 1995).

The importance of adequate Ca supply for N uptake has also been demonstrated in a variety of European liming studies in the field (Huettl, 1989). In addition, reduced N levels in xylem sap of declining Norway spruce trees in Germany have been observed in mature trees – a response that was well correlated with periods of low xylem Ca concentrations and low water potential during the growing season (Osonubi *et al.*, 1988). In the northeastern USA, Ellsworth and Liu (1994) reported a strong linear relationship between foliar N and foliar Ca ($R^2 = 0.64$) in limed sugar maple stands. In southern Sweden, addition of even small amounts of Ca (69 kg ha^{-1}) in combination with $150 \text{ kg of N ha}^{-1}$ and 25 kg ha^{-1} of P increased tree growth by 35% above the N-only treatment. Foliar N concentrations were increased above controls by three times the amount observed in the N-only treatment (Nohrstedt *et al.*, 1993). Such responses are logically linked to two physiological shifts induced by improved Ca nutrition: increased fine-root production, and improved root function as Ca:Al ratios are increased.

(c) *Increased leaching of calcium from foliage.* Leaching of foliar cations by rainfall is a significant component of nutrient cycles of widely divergent forest types. However, it becomes even more important when rainfall exposures are augmented with frequent exposure to clouds, particularly those in industrial regions that have been chemically enriched by strong acids (Lovett *et al.*, 1982). Forests at mountain sites in the eastern USA are exposed to highly acidic mists for from 10% of the time in the north-east (Miller *et al.*, 1993) to 30% in the south-east (Saxena & Lin, 1990). The ionic content of these clouds is typically 10–20 times that of rainfall at the same sites and, as a result, cloud interception by canopies accounts for 20–50% of the total SO_4

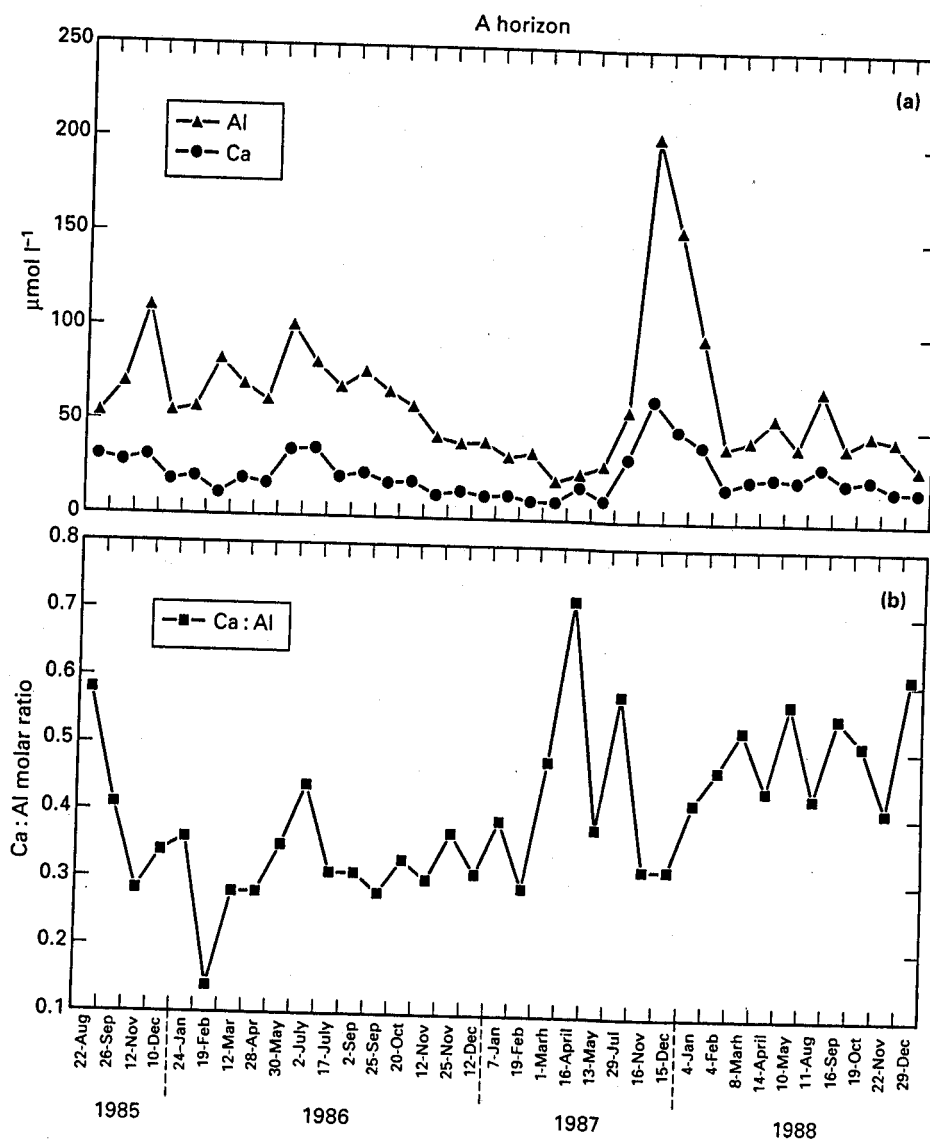


Fig. 11. (a) Temporal variations in concentrations of Al and Ca in A-horizon soil solutions from a high-elevation spruce forest show similar dynamics and chemical dominance by Al, which can interfere with Ca uptake by plant roots. (b) Ca:Al molar ratios remained below the 1.0 level indicative of potential interference of Al with Ca uptake by roots. (original data with coefficients of variation of around 33% from Johnson *et al.*, 1991).

deposited. Enrichment with both SO_4 and NO_x results in the occurrence of occasional mist pH values < 3.0 (Lindberg, 1992) and minimum values around pH 2.5 (Saxena & Lin, 1990). The degree of cation leaching by individual mist events is influenced by mist pH and has been shown to result in losses of up to 36% of the total amount of foliar Ca for red spruce (Joslin *et al.*, 1988).

The loss of Ca from the canopy during rain or mist exposure events represent an intensity change, but one must ask how significant such events can be relative to the larger pools of Ca present in foliage. Experiments with detached twigs indicate that the quantities leached are probably derived from apoplastic supplies and may sum 15% of the total apoplastic cation-exchange capacity at pH 2.1 (Turner & Van Broekheusen, 1992). In Norway

spruce, cation depletion by pH 2.5 mist in epidermal cells was not uniform, but localized in the outer walls (i.e. in the direction of foliar contact (Fink, 1991)). Based on the high concentrations of apoplastic Ca relative to the small physiologically active component of leaf Ca, it has been suggested that apoplastic Ca reserves in conifers are sufficient to compensate for low Ca supply from the soil (Gulpen *et al.*, 1995) thereby reducing the likelihood of Ca deficiency directly limiting Ca supply for forest trees.

Several considerations suggest that maintenance of Ca supply rate at the canopy and whole tree levels are important. First, the concentrations of Ca lost in foliar leaching can be several times larger than the changes in cytoplasmic Ca concentrations associated with physiological and biochemical signaling. Thus

the potential for these events adversely to affect cellular metabolism is high. Second, there is now evidence that important changes in physiological functions are linked to foliar leaching of Ca. Finally, liming studies have conclusively demonstrated improved physiological performance of declining trees, including improved canopy vigor, with improved Ca supply.

(d) *Physiological indicators of altered forest function.* When interdisciplinary teams have investigated the underlying mechanisms of forest responses in areas with high levels of acidic deposition (Huttermann, 1985; Schulze, 1989; Eagar & Adams, 1992; Johnson & Lindberg, 1992), changes in forest nutrient status induced by increased atmospheric deposition of strong mineral acids have been consistently recognized as an important contributing factor to the observed alteration of soil processes and/or physiological responses. From these and many other related studies, increased understanding has been gained of the factors controlling nutrient cycling, and in particular, Ca cycling, in forests. The limited mobility of Ca, its physiological importance, and accelerated loss/immobilization rates with increases in acidic deposition have led to the suggestion that reduced Ca availability could play a primary role in many of the observed physiological responses of forests to acidic deposition (McLaughlin & Kohut, 1992).

In evaluating the effects of acidic deposition on forest ecosystem processes, it has become increasingly apparent that interactions with natural biotic and abiotic stresses are a fundamental component of the expression of forest responses to pollution stress at all levels (Manion, 1981; McLaughlin, 1985; Fuhrer, 1990; Rehfuss, 1991). Here, we examine four measures of response that have typically been associated with the appearance of symptoms of forest decline in recent decades. All have either explicit or potential mechanistic linkages to the known effects of reduced Ca supply, and, in our opinion, all represent areas that are fertile for further research. Each represents a different dimension of Ca control of whole-plant function that is linked to various aspects of Ca metabolism.

An increase in occurrence of winter damage to current-year foliage has been an early and significant visual indicator of stress to red spruce at high elevations in the north-eastern USA (Friedland *et al.*, 1984), and has also been an important theme of diverse research efforts aimed at determining the mechanisms responsible (DeHayes, 1992). In the early stages of recognition of red spruce decline, damage and loss of current-year foliage was found to be closely associated temporally and spatially with the incidence of widespread radial growth declines at high-elevation sites (Johnson *et al.*, 1988). Increased frequency of terminal leader death was also closely associated with radial growth decline (LeBlanc &

Raynal, 1990). The associated growth decline was not attributable to uniquely low temperatures, but rather resulted from an apparent increase in sensitivity of red spruce to winter damage at normal winter temperature (Cook & Johnson, 1989).

A subsequent series of laboratory experiments indicated that foliar exposure to acidic rain and mist was a key component of increased sensitivity. Simulated acid mist-exposure of foliage increased ion leakage from needles and increased the sensitivity of foliage to cold damage (Fowler *et al.*, 1989; Cape *et al.*, 1991). Field studies with mist-exclusion chambers were also used to evaluate the effects of ambient mist/rain on both cold damage and ion leakage of both branches of mature trees (Vann *et al.*, 1992) in the north-east and seedlings in the south-east (DeHayes *et al.*, 1991; Thornton *et al.*, 1994). The results of laboratory and field studies were essentially the same – acid mist-exposure increased sensitivity of red spruce foliage to cold damage. Insights into mechanisms for this predisposition have become increasingly apparent as new techniques for detecting changes in membrane-associated Ca have been developed. In initial experiments with this technique, DeHayes *et al.* (1997) found that changes in membrane-associated Ca were associated with red spruce hardening-dehardening cycles during successive low-high temperature fluctuations, as previously discussed (Fig. 3). Similarly, changes in cytoplasmic free Ca have been found to play a role in cold-shock effects on *Arabidopsis*, a signal that provides the plant with a protective 'memory' against damage from future cold events (Knight *et al.*, 1996).

The linkages of changes in the membrane-associated Ca signal to acidic deposition were further clarified in subsequent experiments by D. H. DeHayes (pers. comm.) that documented a reduction in membrane-associated Ca and a loss in cold hardness for red spruce exposed to mists acidified with H_2SO_4 . The 4–10°C elevation in the temperature threshold for cold damage by pH 3.0 mist exposure, was associated with loss of membrane-associated Ca and membrane destabilization. The physiological significance of these changes led the authors to project increases in susceptibility of montane red spruce to 'low temperature and other stresses that compromise overall forest health'. The significance of a shift in internal status of such a small component of total leaf Ca suggests that total leaf Ca values could have little relevance to the physiological effects of Ca. However, it is apparent that both intensity factors and capacity factors that influence longer-term supply rates are important. Empirical data on Ca influences on carbohydrate metabolism, as well as liming experiments in the field, support the importance of both modes of control.

Foliar cation deficiencies have been an important

into the deeper mineral soil, were produced by acidic mist/rain treatments, and this response was only partially reversed by fertilization with Ca, Mg, or a Ca-Mg combination. Increased needle weight in response to Ca fertilization and evidence of competitive interactions between Mg and Ca were observed in these experiments as well as in fertilization studies with red spruce saplings (Van Miegroet *et al.*, 1993) and mature trees (Joslin & Wolfe, 1994) in the field.

Although physiological responses of red spruce to controlled acid mist/rain exposures closely paralleled physiological changes observed in the field at sites with high frequency of exposure to acidic deposition, the foliar Ca concentrations at which responses were produced in controlled greenhouse studies were approximately twice the foliar Ca concentrations at which dark respiration was enhanced in foliage of saplings in prior field studies (McLaughlin *et al.*, 1993). This led the authors to conclude that observed physiological responses were significantly influenced both by the smaller changes in a physiologically active compartment of foliar Ca that is reduced by acid mist/rain exposure, as well as by total foliar Ca and exchangeable soil supply levels (McLaughlin *et al.*, 1993). This conclusion is compatible with that reached by DeHayes *et al.* (1997) based on direct empirical evidence of the importance of membrane-associated Ca pools.

The importance of exposure-related changes in physiologically active Ca may explain the apparent lack of a strong relationship of Ca levels in soil or foliage with severity of crown deterioration or mortality at a relatively high Ca site, Whiteface Mountain, in the north-eastern USA (Johnson *et al.*, 1994c). The low soil Al concentration, and relatively high soil Ca concentration, which increases with elevation at this site, result in Ca:Al ratios in soil solutions that are only occasionally < 1.0. Ca uptake at this site is relatively high (Johnson, 1992) and no decrease in total foliar Ca (mean concentrations are about 2.2 mg g^{-1}) occurs with increasing elevation in spite of the fact that crown deterioration and mortality increase strongly with elevation (Johnson *et al.*, 1994c). The Whiteface site, because of the advanced state of deterioration of its high-elevation spruce forests, is somewhat difficult to evaluate in terms of responses to nutrient supply. The percentage of dead and declining red spruce trees at this location was quite high, with 100% spruce mortality in 15% of the plots and > 60% foliar loss at 30% of the plots (Johnson *et al.*, 1994c). Such opening up of the canopy, accompanying reduction of foliar area of remaining trees, and the increased return of litter to the forest floor would be expected to have increased Ca supply to the remaining foliage at this site. These conditions, coupled with the high foliar exposure to acidic deposition at this location (Miller *et al.*, 1993), may explain the high variability in foliar Ca

concentrations (ranging from $1\text{--}3 \text{ mg g}^{-1}$; Johnson *et al.*, 1994c) at a site with generally higher Ca supply potential (Fig. 7).

An important component of the decline in health of red spruce at Whiteface Mountain has been the frequent occurrence of winter injury and associated loss of foliage, as already discussed. The repeated loss of a portion of current foliage from winter-damaged shoots can be an important drain on the carbohydrate-production capacity of affected trees. Physiological studies indicate that reduced photosynthetic production associated with low foliar Ca concentrations would exacerbate carbohydrate reductions by winter damage (Amundson *et al.*, 1992).

Sugar maple decline has also been an important issue in regional analysis of forest health in North America (Bauce & Allen, 1991; McLaughlin & Percy, 1999). Although crown mortality and dieback of sugar maple had been reported over extensive areas in Canada, particularly Quebec, by 1982 (Roy *et al.*, 1985), a substantial improvement in health of this species across much of Canada has been reported in most recent surveys (Hall *et al.*, 1997). More detailed spatial and soil chemical analysis of the current condition of sugar maple has revealed that crown dieback within Canada is most apparent on soils with low base saturation and high levels of acidic deposition (Hall *et al.*, 1997).

Mechanistic evidence linking the severity of maple decline to acidic soils with low cation-exchange capacity has become increasingly apparent in recent years (Adams & Hutchinson, 1992). In the weakened upper crowns of declining sugar maple, reduced net photo synthesis (P_n) has been related to reduced foliar Ca and reduced N (Ellsworth & Liu, 1994). In addition, annual basal area growth trends (1978–1987 vs. 1958–1967) of overstory maple in New York and Pennsylvania (USA) were found to be significantly and positively correlated ($P \leq 0.01$) with soil Ca concentrations and negatively correlated ($P \leq 0.05$) with soil Al, H^+ and Fe across a wide range of growth rates (Heisey, 1995). Among foliar nutrients, only foliar Ca ($r = 0.76$; $P \leq 0.01$) and Mg ($r = 0.64$; $P \leq 0.05$) were significantly related to growth of canopy trees. Interestingly, growth of subcanopy trees declined by an average of 56% across all sites in this study regardless of growth trends of canopy trees, which varied widely (–80% to +98%). Negative correlations of subcanopy tree growth rates with soil chemistry were strongest with the acidic cations Al and Fe. Correlations of all variables were weaker overall for subcanopy trees, presumably because of the strong influences of overstory competition on growth of these trees. Under these conditions, correlations of understory growth with soil Mg ($r = 0.59$; $P = 0.05$) and Ca ($r = 0.54$, ns) were positive and stronger than those of N, P or K (the maximum response among these was for soil N

0.85 million hectares) by a systematic network of plots in seven south-eastern states. The very high mortality (within 3 yr of infection), lack of reproduction and rapid spread of the disease of this valuable wildlife forage species led to a series of exploratory studies that we will summarize here because they collectively provide strong inferential evidence suggesting that reduced Ca supply to dogwood would be an important contributing factor to the development of this disease.

First, it should be noted that dogwood is an early successional, Ca-accumulating species, with foliar Ca concentrations approx. six times those of red spruce within the same region. Ca accumulates in dogwood berries, and its nutritionally rich twigs make its fruit a valuable source of Ca for birds and its twigs important forage for browsers (Britton *et al.*, 1997). The occurrence of dogwood anthracnose has been closely linked to those conditions under which Ca uptake from soils would be limited by reduced transpiration (shaded locations, wet coves and northern slopes where temperatures are lower). Dramatic increases in dogwood anthracnose occurred during 1989, which was an exceptionally wet, cool summer in much of the south-eastern USA. Plants in sunny locations showed increased resistance, while those from higher elevations were more sensitive.

Controlled studies suggest that acid deposition can play a role in increasing the susceptibility of dogwood to drought stress as well as increasing its inherent susceptibility to anthracnose (Britton *et al.*, 1997). Exposure to increasingly acidic rainfall within the ambient pH range (5.5, 4.5, 3.5, 2.5) increased post-exposure success of *Discula* infection in experiments repeated on two consecutive years. Rapid equilibration of internal cation status with acidic droplets has been demonstrated as well (Willey & Hackney, 1991), indicating the potential significance of accelerated foliar leaching of Ca. Improved protection of dogwood was provided by fungicidal sprays, and fertilization improved growth, but apparently did not affect resistance. Addition of phosphorous increased sensitivity. Addition of lime reportedly increased resistance (USDA, 1991).

In summary, examination of a wide variety of indicators that characterize variable influences on plant demand relative to Ca supply reveals a pattern of response that is consistent with a significant role for Ca deficiency in the development and/or amelioration of dogwood anthracnose. Based on the collective symptomology, we suggest that reduced Ca supply to dogwood leaves at low soil Ca concentration, low transpiration rates, and/or accelerated leaching of foliar reserves from base-depleted soils reduces the natural resistance of this Ca-requiring species to the infecting *Disculus* fungus. Accelerated foliar leaching of Ca in regions of high atmospheric deposition, particularly in combination with conditions that limit foliar uptake of Ca, may

also be an important predisposing factor that contributes to synchronizing the appearance of foliar stress across diverse sites. We will return later to the issues of justification and research tools to test such speculation at a mechanistic level.

In Poland, Witkowski *et al.* (1987) attributed the rapid development of outbreaks of phytophagous and secondary cambio- and xylophagous insects during the 1980s to increased susceptibility of spruce and fir trees weakened by regional air pollution. These attacks were considered unusual both in the rapidity with which they developed and the fact that trees in the National forests that were affected had been notably resistant to both harsh environmental conditions and diseases in the past.

There are interesting parallels between this European scenario and the development of the Balsam Woolly Adelgid (BWA) on firs in the eastern USA (Timmel, 1986). BWA, a cambial feeding insect with five life stages, can rapidly build lethal populations on infected trees. It was introduced into the northeastern USA in 1907 and BWA developed to epidemic proportions on both balsam fir (*Abies balsamea*) in the northeastern USA and Canada and Fraser fir (*Abies balsamea*) in the southeastern USA in the 1960s (Eagar, 1984). This timing corresponded closely with the onset of reduced growth of mature, high-elevation red spruce at widespread locations in the eastern USA (McLaughlin *et al.*, 1987). Concurrent declines in radial growth of Fraser fir and balsam fir were also noted in the Southeastern USA (Adams *et al.*, 1985). Fraser fir has been particularly susceptible to BWA, and a 1988 survey reported mortality of 44–91% for trees ≥ 12.5 cm dbh over the approx. 26000 ha range of this species in the Appalachian Mountains in the southeastern USA (Dull *et al.*, 1988).

Although the potential role of air pollution in predisposing Fraser fir to BWA was recognized approx. 15 yr ago (Hain & Arthur, 1985), there has been little work on specific mechanisms of resistance since BWA resistance of adult trees was apparent only in limited populations, notably on Mt Rogers at the northern extremity of the range of Fraser fir (Eagar, 1984). Viewed from the perspectives of the role of Ca in disease resistance, evidence of changes in Ca availability to Fraser fir throughout the region, and Ca-related physiological changes to fir documented at field test sites, we believe that several lines of evidence support a more comprehensive evaluation of the potential role of nutrient deficiency in accelerating BWA development in this low-Ca environment.

First, the increased formation of lignin-reinforced compression wood along the bole is now recognized as a defense mechanism for balsam fir (Timmel, 1986) as well as Fraser fir following BWA attack (Hollingsworth & Hain, 1991). Increased lignin formation is a well-recognized mechanism for in-

of increased soil acidification followed by diminished cation availability in response to continued strong anion inputs.

The downward shift in wood Ca at some forest sites in industrialized regions in the past 2–3 decades has often been associated with reduced growth of forest trees (Bauch, 1983; Baes & McLaughlin, 1984; Bondietti *et al.*, 1989; Watmaugh, 1997); however, there are additional implications of such changes. They relate to potential alteration in the structural characteristics of wood formed during periods of reduced Ca supply. Because Ca is so strongly involved in the wood-formation process, and in crosslinking within wood structure, reductions in wood Ca have the potential to affect structural properties as well as the rate and total quantity of wood formed. Studies with red spruce (Bondietti *et al.*, 1989), loblolly pine (*Pinus taeda*, Jordan *et al.*, 1990) and yellow poplar (McClenahan & Vimmerstedt, 1993) have shown that temporal variations in Ca:Al ratios in wood are statistically related to annual variations in radial increment of mature trees. Whether this is directly related to reduced Ca availability at the cambium during wood formation, or to effects on carbohydrate availability for growth, is not known.

Wood structural properties depend to a large extent on the sizes and lengths of xylem cells produced. Controlled studies with smaller trees have demonstrated a wide range of effects on wood formation and anatomy in response to variations in Ca supply rates. In an early study on Ca effects and xylem anatomy of one-year-old loblolly pines (Davis, 1949), cross-sectional areas of Ca-deficient trees were considerably reduced due to reduced proportions of primary tissue of the cortex and the pith. These differences were explained by a decreased number of cells, and to a lesser extent by decreased cell size, under reduced Ca supply. Cambial divisions were obviously most affected by Ca-deficiency. For deciduous trees, Lamb & Murphey (1968) found that Ca deficiency of silver maple (*Acer saccharinum*) seedlings reduced radial growth, which was accompanied by increasing lengths of fibers and vessels. Eastern cottonwood (*Populus deltoides*) responded to increasing Ca with increases in the percentage of fibers and vessels (Foulger *et al.*, 1971), while fiber and vessel dimensions were not sensitive to a change. Paradoxically, Western larch (*Larix occidentalis*) seedlings produced under low soil Ca levels produced 8% more tracheids per unit area, resin canals that were +10% larger and, most significantly, tracheid lengths that increased by +16% (Brady, 1969).

Wood density as an indicator for the total amount of the wall material may increase or decrease in response to altered nutrient supply, and effects of Ca on wood density have been inconsistent. Brady (1969) reported a slight decrease of larch wood

density under low Ca supply while Murphey and McAdoo (1969) reported increased density in *Robinia pseudacacia*. The effectiveness of wood in resisting any particular form of applied force is an additional, more-relevant measure that considers tissue proportions and integrity of lignified cells rather than pure wall material percentages. One measure of the capacity of a material to resist stress is given by the specific stress index (Panshin & DeZeeuw, 1980). Ca deficiency can cause substantial changes in the mechanical properties of wood (Table 3). These changes include reduced strength per unit mass under tension, reduced resistance to rupture, and reduced elasticity, all of which effectively weaken the wood in response to mechanical stress.

Ca can also influence the properties of support tissues, including causing loss of structural integrity of stems of soybean (*Glycine max*; Albrecht & Davis, 1929) and increased brittleness and hardness of pine stems (Davis, 1949). In an exploratory test on individual spruce tracheids, Wimmer & McLaughlin (1996) showed that lignin, Ca and wood mechanical properties (Wimmer *et al.*, 1997) are related to each another. Where low Ca in the xylem tissue was found to produce lower lignin proportions, the hardness and elasticity of secondary wall layers and cell corners were also altered (Wimmer & Lucas, 1997).

Inhibition of lignification of wood is a potentially important consequence of low Ca availability in very acidic soils (Eklund & Eliasson, 1990; Eklund, 1991). Reduced lignification would be more likely in the canopies of larger trees because of both increased transport distances (resistances) and competition for Ca within the sap stream. Reduction in lignin within the crown is of interest because there lignin is widely involved with branch support and in maintaining the mechanical stability of the tree. Maximum lignin contents are typically found slightly below the base of the crown (Jansons, 1966). From the perspective of wood structure, reduced lignin content in the cell walls could lead to a more fragile and brittle xylem wood, thereby increasing the susceptibility of trees to breakage during high mechanical stress periods induced by ice or strong wind. Reduced foliar lignin contents have, in fact, been reported in the upper canopies of oak-maple and pine forests in the Northeastern USA (Wessman *et al.*, 1989). Low lignin contents, which could be detected from spectral data by remote imagery, were well related to low foliar N concentrations and high N mineralization rates in associated soils. In a related study with red spruce examined at multiple sites across the same region, reduced canopy lignin was also found to be significantly related to increased annual N deposition, increased soil N, and reduced Ca and Mg in foliage and in the forest floor (McNulty *et al.*, 1991). Foliar Ca was strongly related to both forest floor Ca ($r = 0.73$; $P \leq 0.01$), and to foliar lignin ($r = 0.66$) suggesting that base cation de-

(+212 kg ha⁻¹ vs. 56 kg ha⁻¹ for SAW) 15 yr after the harvests, inclusion of the Ca removed in residue in whole-tree harvest (approx. 690 kg ha⁻¹) results in a calculated net loss of 478 kg ha⁻¹ of Ca from the whole-tree harvest site, while the SAW site shows a gain of 56 kg ha⁻¹ of Ca, presumably associated with primary mineral weathering.

An interesting feature of this study was the trend towards increasing Ca levels in the upper 15 cm of soils of all treatments (unharvested (+175%), SAW (196%) and whole-tree harvest (48%)) at the end of the 15-yr interval. Increasing C and N levels in upper soils of all treatments suggests that mineralization, uptake and rooting were active resupply mechanisms to the upper soil profile during the 15 yr interval. Despite the increases in apparent Ca availability in the shallow soils following whole-tree harvest, relative availability of exchangeable Ca was lower in both A (-56%, $P \leq 0.01$) and E (-54%, $P \leq 0.10$) soil horizons for whole-tree harvest vs. SAW. The reduced relative availability of Ca with the whole-tree harvest treatment was supported by foliar Ca concentrations that were lower in whole-tree harvest by an average of 27% for six species compared, but Ca levels were above whole-leaf deficiency levels. Thus, while whole-tree harvest resulted in increased Ca losses from this site, rates of resupply of exchangeable Ca by mineralization and uptake from the dolomitic parent material were apparently high enough to resupply surface soils during the initial 15 yr following this harvest cycle.

Over longer-term cycles, repeated harvesting can also be evaluated as a soil-acidifying process associated with the net removal of cation bases from the soil (Ulrich & Matzner, 1986). The cation removal rate for conventional bole harvest in a German Norway spruce forest was estimated to be equivalent to addition of 0.5–1.0 keq ha⁻¹ yr⁻¹ of acidity, and approximately to balance natural mineralization rates. For spruce and beech forests, branch removal increased base cation losses by 100% and 20% over bole-only removal and represented a significant increase in soil acidification, particularly when coupled with high rates of acidic deposition (Ulrich & Matzner, 1986). Knowledge of the relative importance of weathering, leaching and uptake rates by vegetation coupled with estimates of the pools in foliage and forest floor can provide forest managers important insights into long-term sustainability of nutrient cycles with available harvesting options. Those choices appear to be particularly important for Ca because relatively rapid cycling times and potential system losses following harvest are important processes in uptake and retention of Ca.

(b) *Managing forest nutrient supply.* Understanding of the physiological implications of base cation deficiency has been strongly reinforced by experience with liming and fertilization experiments in Central

Europe. Review of the role of natural and induced nutrient deficiencies and the effects of liming and fertilization as remediation tools (Zoettl & Huettl, 1986; Huettl, 1989; Kreutzer, 1995) has provided many insights into the physiological basis of the effects of Ca on forest growth as well as important insights into effects on soil chemistry and litter decomposition processes. Such studies have also been important in evaluating interactions among Ca and other nutrients, most notably N. Huettl (1989) summarized the results of numerous fertilization and liming experiments designed to remedy nutrient imbalances due to 'accelerated soil acidification and increased nutrient leaching from the canopy ... that demonstrated a fast and sustained revitalization of declining forest ecosystems'. Among the evidence of recovery following liming was increased nutrient uptake, including N, increased vigor of forest canopies, improved growth, resistance to frost damage, and enhanced production of fine roots. Changes in rooting patterns associated with distribution of Ca to deeper soils have been reported as roots follow liming effects into deeper soil profiles (Huettl, 1989). Such changes are of particular interest in trying to understand the effects of liming on both forest nutrient uptake and growth.

Perhaps most interesting are the effects of liming on N uptake and metabolism and litter decomposition rates (Kreutzer, 1995). Heavy liming (30 t ha⁻¹) has been shown to reduce N₂O emissions to the atmosphere by 75% from N-saturated soil in the high deposition (N and S) Solling area in Germany (Brumme & Beese, 1992). Five years after lime application, the pH of the humus at this site was raised from 4.5 and 6.5, and CO₂ emissions were increased by 31%, suggesting that microbial decomposition rates and, perhaps, root growth, had increased. However, soil solution nitrate concentrations were not increased by liming in this study, and the authors suggest that a shift to greater N₂:N₂O in the emission gases may have occurred, indicating liming-induced alteration of N metabolism. High N₂O emissions from N-saturated forest soils are considered a consequence of reduced efficiency of N utilization associated with chronic high inputs of atmospheric N (Goulding *et al.*, 1998). With liming at modest levels, increased N uptake and retention are also likely as the zone of rooting activity changes and total root mass is increased.

In one of the few long-term liming studies designed to ameliorate the effects of acidic deposition in the USA, liming was found to increase significantly basal area growth and improve the crown condition of overstory sugar maple in Pennsylvania (Long *et al.*, 1997). Foliar Ca concentrations doubled, Mg quadrupled, and seed yields were increased in these tests with dolomitic limestone addition at 22 Mg ha⁻¹. Interestingly, growth

VI. CONCLUSION

1. Whole-tree perspectives

In this article we have sequentially reviewed some fundamental aspects of Ca regulation of basic physiological processes at cellular, organ and organismic levels. In addition, we have examined the biogeochemical basis of Ca effects on some ecosystem processes. Wherever possible we have sought to relate mechanisms of action to biological control of processes at the whole-plant and whole-system level. As one moves up through successive organizational scales, the diversity of influences of multiple nutrients and multiple chemical and biological stresses on plant and ecosystem function are increasingly apparent and conclusions regarding specific influences of any single influence are less direct. However, Ca occupies a unique physiological niche in the diversity of its influences on plant function and, for this reason, inferences regarding Ca regulation of forest processes can be examined from many dimensions.

At the cellular level, the interconnected apoplastic network of cell walls and cell membranes surrounding cytoplasm that functions at three orders of magnitude lower Ca levels has some interesting analogies to the neurological system of animals. Electrochemical signals sent through this system in multiple directions appear to play a role in local and more remote defense against pathogens and physical and chemical environmental stresses, such as leaf cation exchange, low temperatures and cell wall

damage. Environmental signals that change the timing and distribution of Ca fluxes and pool sizes in the plant play an important role in biological responses at multiple levels and some of these are summarized in Table 4. It can be seen from this table that the internal signals that provide biochemical control, typically in the order of a few mg l^{-1} , are relatively small compared to chemical signals provided by foliar leaching from the atmosphere and the temporal variations in Ca concentrations supplied from the soil solution through xylem transport.

It is not now clear how plants integrate the influences of variable Ca supply imposed by the more stressful and more variable soil chemical environment encountered under increasingly acidic soil conditions into the control of physiological processes. It is now apparent, however, that increased foliar leaching of Ca can have adverse physiological effects that are not readily reversed or compensated by local (membrane) resupply. Many liming experiments now verify the positive effects of increased soil Ca supply rates on improved canopy function and structure, indicating that increasing Ca supply rates can reduce or eliminate symptoms of physiological stress in the upper canopy.

Important roles for Ca have now been identified in the following areas: the structure and function of membrane systems that regulate nutrient flow through roots and both water and solute fluxes through leaf membranes; stomatal control of water flux from foliage; activation of enzyme systems that are involved in cell wall synthesis and in providing physical and chemical defense against injury and

Table 4. Comparative strengths of fluxes and signals involved in calcium supply and regulation of some physiological and ecological processes in terrestrial vegetation

Compartment	Content	Signal*	Species/conditions	Reference
Rainfall	440 $\mu\text{g l}^{-1}$	440 $\mu\text{g l}^{-1}$	Deciduous, USA/TN	Johnson <i>et al.</i> (1985)
Cytoplasm	0.04–400 $\mu\text{g l}^{-1}$	20 $\mu\text{g l}^{-1}$	General range	Bush (1995)
		2 $\mu\text{g l}^{-1}$	Stomatal guard cell	McAinsh <i>et al.</i> (1996)
		38 mg l^{-1}	<i>Arabidopsis</i> cold shock	Knight <i>et al.</i> (1996)
Cell walls, membranes	40 mg l^{-1}		Red spruce seasonal	DeHayes <i>et al.</i> (1997)
Foliage	1.5–2.5 mg g^{-1}		Red spruce field	Robarge <i>et al.</i> (1989)
	> 3.0 mg g^{-1}		Fraser fir field	
Lechate net flux		800 $\mu\text{g l}^{-1}$	General forests	Cape <i>et al.</i> (1990)
		100 $\mu\text{g l}^{-1}$	Red spruce pH 3.0	DeHayes <i>et al.</i> (1998)
			Red spruce pH 5.0	DeHayes <i>et al.</i> (1998)
Xylem sap	48 mg g^{-1}		Healthy Norway spruce	Osonubi <i>et al.</i> (1988)
	36 mg l^{-1}		Declining Norway spruce	Osonubi <i>et al.</i> (1988)
Xylem cell walls	0.5–1.0 mg g^{-1}		Red spruce	Bondetti <i>et al.</i> (1990)
Root cortex	0.5 mg g^{-1}		Norway spruce	Godbold <i>et al.</i> (1988)
	0.1–1.6 mg g^{-1}	0.01–0.06 mg g^{-1}	Beech cross section	Hult <i>et al.</i> (1992)
Soil solution	2.4 mg l^{-1}	0.4–24 mg l^{-1}	Beech (Solling, Germany)	Matzner & Prenzel (1992)
			10 cm depth	
	4 mg l^{-1}	3–15 mg l^{-1}	Spruce (Solling, Germany)	Matzner & Prenzel (1992)
	0.40–2.6 mg l^{-1}	0.4–6.0 mg l^{-1}	Spruce USA/SA	Johnson <i>et al.</i> (1991)

*Signal is defined as a change in content (an estimate of typical levels from cited sources) produced by changing environmental and/or physiological conditions.

would expect such modifications to be more evident in later successional stages of forest development when increasing amounts of system Ca are tied up in woody stems.

Studies of the effects of acidic deposition on forested watersheds have added significantly to current understanding of the importance of reduced Ca supply to forest system structure and function. Among the observed responses are reduced size and distribution of root systems; increased mortality rates of mature trees from amplifying natural stresses, such as winter injury; and loss of upper-canopy integrity. Additional studies in Europe indicate that, with continued Ca loss, reduced flow of Ca through food chains can adversely affect the reproductive success and skeletal integrity of some bird species (Graveland, 1996). Interestingly, birds, like plants, have no capacity to store Ca and thus Ca supply at the time of nesting is critical to eggshell integrity and nesting success.

The results of liming studies indicate that increasing Ca supply in Ca-depleted systems can have very positive effects on the vitality of both soil and vegetation. Included among the positive effects are improved fine-root production and root distribution, an important factor in responses of forests to drought, nutrient uptake and belowground competition. However, it is apparent that additional research is needed on liming application rates and particle sizes to optimize Ca supply with time and soil depth. Over-liming and use of strong anion carriers can have negative effects on decomposition and root distribution that must be recognized and avoided. In many types of forests, a significant fraction of total available Ca is contained in above-ground biomass, making management of forest residues a particularly critical issue to maintaining longer-term Ca supply.

Interrelationships between Ca and N can be expected to become increasingly important in future forest growth and nutrient cycling. In Europe, patterns of increasing basal area growth in many areas have followed the widespread foliar symptomatology and evidence of declining forest vigor apparent in the late 1970s and early 1980s (Schadauer, 1996; Sterba, 1996; Pretzsch, 1996). In contrast to improving growth at lower elevations, reduced height and volume growth trends have continued in the mountains of Central Europe (Pretzsch, 1996). Several factors must be considered in relating growth rates in the 1980s to previous and potential future growth. These include improving climatic conditions in the 1980s following the very dry 1970s, implementation of improved silvicultural techniques over the past 30 yr, and increasing N supply from atmospheric deposition, which has had its most positive effects on stands with lower initial N levels (Schadauer, 1996). Another biological factor is the improved genetics of managed-stand and

natural selection for stress resistance within unmanaged stands. Superimposed on this has been the significant reduction in pollution stress from reducing SO_4 deposition in the last two decades. Sulfur emission decreased between 1981 and 1991, amounting to 67% in western Germany and 35% in eastern Germany, which began its reduction program later in the decade (Meesenburg *et al.*, 1995). European reductions in SO_4 and base cations have been 29% and 39%, respectively, for The Netherlands, and 63% and 27% in Sweden (Hedin *et al.*, 1994). In north-west Germany, where deposition of SO_4 has decreased by 50–70%, N deposition has remained at about the same levels, and Ca deposition has decreased. The net effects of changes in these multiple factors on soils at Solling, a site heavily impacted by deposition in the past, has been a 55–60% reduction in exchangeable base cations during the past two decades (Wesselink *et al.*, 1995).

For many reasons, it is difficult to make confident projections of future forest growth patterns in Europe (Sterba, 1996). However, from the mechanistic approach we have presented here, it is useful to consider how whole-tree nutrient supply patterns for Ca might be expected to respond to the shifts in growth and deposition patterns discussed. In brief, canopy processes, both Ca absorption from deposition and foliar Ca leaching from acidic deposition, should be significantly reduced in importance due to reduced emissions of S. Reduced S deposition would also be expected to reduce Al concentrations in soil solutions, a response that has been observed at the Solling Forest (Meesenburg *et al.*, 1995). As a consequence, competitive interference of Al with Ca uptake would be expected to be reduced. However, reduced atmospheric inputs of Ca, which in some areas are comparable to reduced SO_4 deposition, can be expected to diminish the effectiveness of reduced S inputs on Ca cycling (Likens *et al.*, 1996). By contrast, the continued high N deposition rates, which in north-west Germany significantly exceed forest uptake, can be expected to continue to mobilize and deplete Ca and Mg by leaching and accelerated sequestration in growth. N-fertilization experiments in Sweden suggest that increased forest growth in response to high N loading occurs at the expense of reducing carbohydrate allocation to root systems (Persson & Majdi, 1995). At some point, reduced Ca supply would be anticipated to limit growth and N uptake in soils with lower base saturation and to accelerate processes that lead to N saturation.

In Sweden, results of mapping base cation: Al ratios in soils and critical loads of acid deposition suggest that over 80% of forests currently receive acidic deposition in excess of present critical loads, and growth reductions have been predicted (Sverdrup *et al.*, 1992). However, Binkley and Hogberg (1997) have cautioned against applying

an improved capacity to use this knowledge to protect and enhance forest growth and forest ecosystem integrity.

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